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Low salinity habitat use patterns of southern flounder (*Paralichthys lethostigma*) on the Texas Gulf Coast

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Low salinity habitat use patterns of southern flounder (*Paralichthys lethostigma*) on the Texas Gulf Coast

by

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Dedication

This work is dedicated to my father, Douglas Nims, for teaching me to always work hard and ask lots of questions, and for fostering and supporting my love for nature by taking me on as many fishing trips as possible.

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Abstract

Low salinity habitat use patterns of southern flounder (*Paralichthys lethostigma*) on the Texas Gulf Coast

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Southern flounder (*Paralichthys lethostigma*) populations have declined over the last 25-30 years throughout its range. With this rapid decline, the sustainability of the southern flounder fishery and population viability of this commercially and recreationally important fish has come into question. Previous research conducted in the Northern Gulf of Mexico and North Carolina, has shown that southern flounder often reside in freshwater for significant periods of time during the juvenile life history stage. Juvenile southern flounder have been collected at salinities below 10 in Aransas Bay (TX), suggesting that Texas southern flounder might also have critical periods of freshwater residency. However, the presence of a low salinity residency period in southern flounder in Texas has not previously been tested. Patterns of low salinity residence were determined using otolith microchemistry, using Ba/Ca ratios to determine movements across salinity boundaries. Water samples were collected from the major tributaries to the area in order to establish the Ba/Ca freshwater signature. Otolith Ba/Ca values revealed a high degree of variability in habitat use patterns among individuals. The mean percent time that an individual

spent in low salinity habitat was skewed toward the lower end (15%) but a significant proportion of the individuals sampled (59%) used low salinity habitat at some point during their life. The remaining individuals (49%) never entered low salinity habitat. This work indicates that there are two distinctly different groups of habitat use patterns in the population. This work demonstrates that southern flounder in Texas exhibit different habitat use patterns from their congeners in North Carolina and the Northern Gulf of Mexico and can help contribute to the spatial management of the southern flounder population on the Gulf Coast of Texas.

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LOW SALINITY HABITAT USE PATTERNS OF SOUTHERN FLOUNDER (*PARALICHTHYS LETHOSTIGMA*) ON THE TEXAS GULF COAST

Introduction

The study of contingents, or groups within distinct populations that demonstrate alternate migratory behaviors or habitat use patterns (Secor 1999), represents a significant shift in the understanding of fish migration (Secor 2010). Contingents can be distinct such that one will be dispersive (migratory), while the other remains resident, or contingents may differ in the timing, frequency, and duration of habitat use. In addition to differential migration patterns and habitat usage, contingents may exhibit differences in the timing of spawning of cohorts or differences in growth rates (Kerr and Secor 2010). Although populations may demonstrate spatial structuring, contingents may not be genetically distinct.

Understanding contingents within a population is critical because distinct contingents can mitigate detrimental effects on fisheries populations. If there is an event that adversely impacts one contingent (e.g. environmental events, disease, anthropogenic impacts, etc.), other contingents may not be affected (Secor 1999). Depending on the species and environmental conditions, contingents and their structure may result in differences in the stability, resilience, and productivity of the population (Kerr et al. 2010). Additionally, contingent structure may support overall population persistence (Kerr et al. 2010). Identification of contingents within populations is an important step in developing fisheries conservation strategies, as the ecological consequences of contingents could have significant impacts on population dynamics. To successfully rehabilitate a fisheries species, knowledge about what habitats a particular contingent is using must be expanded (Beck et al. 2001, Secor 1999).

Partial migration is a specific type of contingent behavior (Kerr et al. 2009, Secor 2010). Partial migration, as defined by Chapman et al. (2011), “occurs when a population of animals contains both migratory and resident individuals.” Partial migration is a widespread phenomenon in fisheries, with the existence of ecologically important contingents identified in a number of species, including white perch (*Morone americana*), many species of Salmonidae, anguillids, striped bass (*Morone saxatilis*), artic char (*Salvelinus alpines*), and Atlantic cod (*Gadus morhua*) (as reviewed in Secor 1999 and Kerr et al. 2009). Partial migration is a useful and widely applicable framework with which to examine fish populations, as it provides a mechanistic understanding of the life cycle diversity within a population and the evolution of such behavior (Kerr et al. 2009, Berthold 2001). Understanding partial migration is critical because migratory behaviors have important impacts on the population dynamics of a species. There are many hypothesized processes that govern migratory behavior, with the majority of evidence indicating that partial migration is maintained throughout evolutionary time mainly as a conditional strategy, implying that individuals chose an alternative migratory strategy based upon a fixed intrinsic state or a plastic extrinsic state (Chapman et al. 2011). For example, growth rate in the early life stages has been identified as an important factor in determining migratory behavior in several fish species (as reviewed in Kerr et al. 2009). In other populations, sex is the primary factor variation in migratory syndromes (Jonsson & Jonsson 1993). Understanding partial migration is the focus of continued ecological research, with a multitude of studies striving to determine the ecological drivers and the consequences of partial migration. As many species worldwide fall into decline, understanding the mechanisms governing partial migration becomes critical to conservation (Wilcove and Wikelski 2008).

In addition to understanding the mechanisms driving partial migration, it is critical to investigate the ecological consequences of partial migration. A recent study concluded that partial migration of cyprinids from lakes to streams had a significant effect on lacustrine trophic dynamics: winter mean size of zooplankton increased with an increased number of fish migrating from the lake and peak biomass of phytoplankton was achieved with a higher number of resident fish (Brodersen et al. 2011). Additional studies have indicated that partial migration has important influences on trophic structure and stability (Brodersen et al. 2008, Post et al. 2008).

One way to identify ecologically important contingents in a population is through the use of otolith microchemistry (Campana 1999; Elsdon et al. 2008). Otoliths are $CaCO_3$ structures located in the inner ear of a fish that aid in hearing and balance. These structures are metabolically inert, making them reliable indicators of a fish's migration history throughout its lifetime (Campana and Thorrold 2001). Otoliths are a particularly useful tool for establishing the movement patterns of fish because they act as a natural tag by incorporating some elements in proportion to their dissolved abundances in the surrounding water (Bath et al. 2000; Walther & Thorrold 2006). Several chemical proxies vary predictably across salinity gradients, including stable isotopes ($^{87}Sr/^{86}Sr$) and some elemental ratios (Sr/Ca, Ba/Ca). Assuming the isotopic and elemental signatures of endmembers can be established, these ratios can be used to establish movement patterns across salinity gradients for individual fish (Kraus and Secor 2004, Kennedy et al. 1997, Elsdon and Gillanders 2005, McCulloch et al. 2005). Sr/Ca has previously been used to identify estuarine habitat use of fish because the marine value of Sr is very high compared to the Sr concentration of most freshwater and estuarine habitats (Brown & Severin 2009; Phillis et al. 2011). Conversely, Ba/Ca ratios are generally higher in fresh water and lower in marine waters. As a result, there are inverse relationships between Sr:Ca and Ba:Ca across the salinity gradient for most coastal systems. These elemental ratios can be used in combination to reconstruct movements of fish between fresh and marine habitats (McCulloch et al. 2005; Walther et al. 2011). Seasonal variation in freshwater endmembers, caused by flow rates or suspended sediments, along with the physiological processes and elemental partitioning involved in the otolith incorporation process means that sometimes using a single tracer to interpret habitat use patterns can be limiting (McCulloch et al. 2005). Therefore, multiple tracers are often used as a verification method (McCulloch et al. 2005)

Southern flounder (*Paralichthys lethostigma*) is a highly sought after flatfish, both recreationally and commercially, along the Texas Gulf Coast. In the past 25 – 30 years, southern flounder populations have declined sharply throughout the Gulf of Mexico, most likely due to anthropogenic causes (GSMFC 2000). With this rapid decrease in population sizes, the viability of this important benthic predator has come into question. The importance of estuarine habitat to southern flounder juveniles has previously been established (Glass et al. 2008, Nañez James et al. 2009). However, research conducted in North Carolina (Burke et al. 1991) and the northern Gulf of Mexico (Lowe et al. 2010, Reichert and Van der Veer 1991, Rogers et al. 1984), indicates that both juvenile and adult southern flounder are sometimes present in fresh water. It remains unclear whether there is a dispersive contingent of juvenile southern flounder utilizing low salinity ($\leq$5) habitat in Texas.

Previous research has shown that the abundance of one contingent of migrants is dependent upon the other group, as their dynamics are coupled through density dependent effects (Griswold et al. 2011). Therefore, identifying and understanding the contingents of a population that display different migratory behavior is critical to understanding the population dynamics of a particular species. Variation in habitat usage among contingents could result in significant fluctuations in the population dynamics of southern flounder. Southern flounder are already a highly migratory species, inhabiting the bays and estuaries for most of the year before moving out into the open ocean to spawn (Wenner and Archambault 2005, Stokes 1977). In the case of southern flounder, the dispersive behavior being investigated is when juvenile southern flounder are using low salinity habitat, while their congeners remain in the marine or estuarine environment. In the case of southern flounder, the dispersive behavior is possibly occurring during one of the most critical phases of an individual's life history, the juvenile phase. Investigating the migratory behavior will reveal important information about the population dynamics, which will lend itself to more effective and comprehensive conservation of this species. All animals are susceptible to increasing anthropogenic activity, but habitat fragmentation and global climate change have a disproportionately large impact on migratory species (Wilcove and Wikelski 2008). As freshwater and low salinity habitats are among the most highly impacted habitats, it is important to understand how juvenile southern flounder use this particular type of habitat.

CHAPTER 1: INVESTIGATING PARTIAL MIGRATION OF SOUTHERN FLOUNDER ON THE TEXAS GULF COAST

Introduction

In the Gulf of Mexico, southern flounder (*Paralichthys lethostigma*) represents one of the most sought-after fisheries species, both commercially and recreationally. Over the last 25-30 years, southern flounder have suffered a decrease in population sizes throughout its range, which reaches from North Carolina to the lower east coast of Florida and from the southwest coast of Florida, along the Gulf Coast to northern Mexico (GSMFC 2000). Since peaking in 1987, both recreational and commercial landings of southern flounder have decreased in Texas (GSMFC 2000). From 1975 – 2008, the long-term decline in juvenile southern flounder abundance was estimated at 1.3% per year, while adult southern flounder populations have declined at 2.5% per year in Texas (Froeschke et al. 2011). This rapid rate of decline has given rise to questions about the continued viability of the southern flounder fishery.

Throughout the Gulf of Mexico, southern flounder has historically supported an extensive commercial and recreational fishery. Southern flounder and gulf flounder (*Paralichthys albiguta*) are the dominant flatfish harvested both commercially and recreationally in the Gulf of Mexico (GSMFC 2000), with southern flounder representing over 95 percent of harvested flounder in Texas (Riechers 2008). Over the past 25 – 30 years, there have been marked decreases in both the commercial and recreational landings of southern flounder (GSMFC 2000, Reichers 2008). Inshore commercial harvest peaked at 500,000 fish per year from 1985 – 1987 and has since declined to less than 100,000 fish (Reichers 2008). Offshore commercial harvest of southern flounder has experienced a similar decline: from 325,000 fish in 1987 to less than 50,000 fish in 2007 (Reichers 2008). Recreational catches have also fallen from 200,000 fish in 1987 to less than 50,000 fish (Reichers 2008). Compounding the problem of declining stocks is the marked increase in the nominal ex-vessel prices for southern flounder in the same time frame (GSMFC 2000). In recent years, the Texas Parks and Wildlife Department (TPWD) has implemented several measures to mitigate the detrimental effects of fishing pressure on southern flounder populations. For example, TPWD has instituted a ban on gigging during the month of November, during the peak of the spawning migration. Additionally, TPWD has decreased bag limits from 10 fish to 5 fish and is in the beginning stages of implementing a restocking program. Additionally, measures were taken to reduce by-catch of southern flounder juveniles and adults in the shrimp trawling industry.

The ecological impacts of the decline of large marine predators have been well documented (as reviewed in Heithaus et al. 2008). Top predators influence community dynamics by directly (e.g. by inducing mortality) and indirectly (e.g. by influencing the behavior of other species) (Heithaus et al. 2008). Therefore, a decline in a top predator can dramatically impact an ecosystem by impacting other trophic levels and even other fisheries (Heithaus et al. 2008). Additionally, evidence indicates that top-down effects (primarily indirect consumer effects) are the key drivers of coastal benthic ecosystem structure and function (Heck and Valentine 2007). Declining stocks of southern flounder are also troublesome because, as a benthic carnivore at the top of the food chain, southern flounder fill an important ecological niche (Wagner 1973). Younger southern flounder eat plankton, small invertebrates, small fish, and mysids. Older southern flounder prey on mostly fish, including anchovy (*Anchoa* spp.), mullet (*Mugil* spp.), menhaden (*Brevoortia* spp.), and Atlantic croaker (*Micropogonias undulatus*) (Stokes 1977). Older fish will also consume crabs and shrimp (Stokes 1977).

As a valuable fisheries species, much of the reduction in southern flounder stocks has been attributed to overfishing. However, other factors, such as habitat alteration and degradation, bycatch of juvenile southern flounder in the shrimping industry, freshwater diversion, and rising water temperatures, are also detrimental to southern flounder populations (GSMFC 2000). Identifying critical habitat that is vital to the persistence of the fishery is a key factor in effectively managing and maintaining the southern flounder fishery in the Gulf of Mexico (GSMFC 2000).

Southern flounder are particularly vulnerable to habitat destruction because of the variety of habitats that individuals can occupy throughout their life history. Adult southern flounder migrate from the bays and estuaries to offshore locations to spawn from October through January, with peak spawning migration typically occurring in November (or when the temperature declines 4°-5° C) (Stokes 1977). Eggs, which are buoyant and pelagic, hatch offshore and larvae are returned to the bays and estuaries via currents. Approximately 35 days post-hatch, larvae complete metamorphosis and settle into benthic habitats (Wenner and Archambault 2005). This can occur anytime between January and April (GSMFC 2000).

While the habitat preference and distribution of southern flounder changes seasonally and according to life history stage (GSMFC 2000, Glass et al. 2009, Nanez-James et al. 2009), it has been traditionally thought that juvenile southern flounder remain in the bays and estuaries until maturity, when they will partake in the spawning migration to the ocean (Wenner and Archambault 2005). In the Aransas-Copano Bay system, newly settled juvenile southern flounder are found in higher abundance close to tidal inlets in vegetated sandy areas, as compared to non-vegetated muddy bottom habitat that is located further from tidal passes

(Nañez-James et al. 2009).Throughout their range, southern flounder juveniles and adults have previously been reported in salinities from 0 to 60 (GSMFC 2000, Stokes 1977, Rogers et al. 1984, Tagatz 1967). Physiological adaptation to salinity tolerance changes with age (GSMFC 2000). Southern flounder larvae have been found to have a decreased tolerance to lower salinities (Daniels et al. 1996, Moustakas et al. 2004) but as fish mature, their salinity tolerance increases (Smith et al. 1999). Post-metamorphosis juveniles exhibit statistically significantly lower survival at a salinity of 0, as compared to salinities ranging from 5 – 30, while older juvenile flounder (95.2 mm total length (TL)) have been demonstrated to have 100% survival when held at salinities ranging from 0 – 10 (Smith et al. 1999). Survival of juvenile southern flounder 20 – 24 mm TL was significantly lower when juveniles were abruptly transferred from full seawater to a salinity of 0.5, than from seawater to a salinity of 10 (Rawlinson 2009). Adult southern flounder have been sampled in fresh water and very low salinities (Gilbert 1986, Daniels 2000, Wenner and Archambault 2005). In Texas specifically, southern flounder have been reported in salinities as low as 6 (Stokes 1977). However, the importance of low salinity habitat to southern flounder populations in Texas has not been systematically investigated.

Recent research has revealed the substantial use of freshwater habitat by juvenile southern flounder. Lowe et al. (2010) demonstrated that during the juvenile phase, southern flounder in the Mobile-Tensaw Delta, Alabama, spend a significant portion of their lives in fresh water. In this study, 68% of individuals studied were hatched in high salinity waters and subsequently moved to freshwater habitat. The remaining individuals moved into freshwater habitat so quickly after hatch that there was no marine signal detectable in the otolith core, which contradicts the conclusions of previous studies about the salinity tolerance of larval southern flounder. This study highlights the fact that there are differing habitat use patterns in southern flounder and indicates that there may be southern flounder populations that exhibit partial migration.

There are several reasons why it is important to investigate if there is a dispersive contingent present in Texas southern flounder populations, and therefore if southern flounder populations in Texas exhibit partial migration. Due to the frequency of drier conditions in Texas, it is possible that juvenile southern flounder may not have freshwater or low salinity habitat regularly available to them. Adequate freshwater inflow has been established as an important factor in the distribution and abundance of many important fisheries species, such as black drum (*Pogonias cromis*), and blue crabs (*Callinectes sapidus*) along the Texas Coast (Longley 1994). Additionally, freshwater inflows are important to primary prey items of southern flounder (Longley 1994). Estuaries have previously been established as an important nursery ground for juvenile southern flounder (as reviewed in GSMFC 2000), but it is possible that freshwater inflow might also affect growth, survival, and performance of juvenile southern flounder. In response to the decline in southern flounder stocks, the Texas Department of Parks and Wildlife has put into place various measures in recent years, such as decreased bag limits and a ban on gigging during the month of November, and initiated the development of a stocking program. However, in order to develop comprehensive management plans, a better understanding of migratory behaviors of the juvenile life stage is critical. Additionally, it is especially important to determine the relevance of low salinity habitat for sustaining southern flounder populations, as precipitation to the region is projected to decline in the region over the next 100 years (IPCC 2007). Maintaining minimum environmental flows is critical to maintaining the integrity of an estuary (Richter 2010), as adequate freshwater inflow is necessary for the delivery of nutrients and sediments to the estuary, physical mixing processes, and the filtration services that estuaries provide (Richter 2010, Longley 1994). As many fish species, and their prey items, rely on the estuary for nursery habitat, the maintenance of the integrity of the estuary has a large impact on coastal fisheries (Longley 1994). Although freshwater inflows are also dependent on precipitation patterns, freshwater inflow is heavily impacted by anthropogenic alterations to the watershed, including the construction of dams and other watershed alteration (Richter 2010, Longley 1994).

Partial migration is often driven by density-dependence, differential growth rates, or sex (as reviewed in Kerr et al. 2009, Jonsson and Jonsson 1993). Previous studies have demonstrated that the highest number of southern flounder juveniles was found in freshwater conditions and as residence time and growth increased, individuals were more prone to move into higher salinity waters (Rogers et al. 1984). Density and condition of newly settled southern flounder has been found to vary significantly within a system, indicating that southern flounder settlement can be driven by density dependent effects (Glass et al. 2008). This study will investigate the relationship between growth (as measured by otolith accretion) during the first year of life and how this impacts low salinity habitat use.

One of the difficulties of using otolith microchemistry to reconstruct the salinity environment experienced by a fish over its lifetime is that it is difficult to determine whether the fish chose to move into an environment with a different chemical makeup or if the fish stayed in the same location while the water changed around the individual. In other words, if otolith microchemistry reveals that juvenile southern flounder are making use of low salinity habitat, was this residence in low salinity water an active choice or a product of changing water flows around the fish? Determining whether low salinity habitat use is an active behavioral choice is also necessary to understanding the importance of low salinity habitat to juvenile southern flounder.

Southern flounder in Texas may exhibit patterns of low salinity habitat use distinct from southern flounder from North Carolina and the Northern Gulf of Mexico, as other notable disparities between these populations have previously been reported, including significant genetic differences (Blandon et al. 2001) and variations in the timing and critical temperature of sexual differentiation (Luckenbach et al. 2003, Luckenbach et al. 2005, Montalvo et al. 2010).

This study demonstrates the importance of low salinity habitats to southern flounder in south Texas, further highlighting the importance of maintaining adequate freshwater inflows into the estuary. Since the presence of contingents in a population contributes to the long-term stability of a population (Kerr et al. 2010), determining the differing patterns of habitat use during the juvenile period of southern flounder can result in more consideration of the spatial management of habitat that is critical to juvenile southern flounder growth and development. Determining if southern flounder in Texas exhibit partial migration will provide insight into population dynamics and help to develop more effective conservation measures for this ecologically important species. This study provides evidence of divergent migratory strategies during the juvenile phase of southern flounder on the south Texas Gulf Coast.

Materials and Methods

Water chemistry

Otolith microchemistry can only be used to assign fish to a specific habitat if the chemical composition of the location is known. Therefore, a water sampling survey of tributaries to the south Texas Gulf Coast was conducted in 2010 and 2011. Water samples were collected in July and August of 2010 and August of 2011 from the Nueces River, Oso Creek, the Mission River, the Aransas River, Copano Creek, the Guadalupe River, and the San Antonio River (Figure 1.1, Appendix A). Young-of-year southern flounder are juveniles at this time, so sampling during this period gives an accurate representation of the chemical environment that juvenile southern flounder would be experiencing if they are utilizing low salinity habitat.

Samples were collected at three points along each tributary in order to constrain spatial variability in the freshwater endmember. Samples were collected during 2010 and 2011 to assess interannual variability in elemental composition. During 2010, samples were collected in duplicate at each location. Analysis indicated that replicates were not significantly different from each other; therefore, only one sample was collected at each location in 2011. In 2011, additional samples were collected in the Nueces River, so that mixing curves of elements and isotopes across the entire salinity gradient (0 – 40) could be quantified. Water samples were collected using acid-washed polytetrafluorethylene (PTFE) syringes and filtered with PTFE 0.45 μm and 0.20 μm filters. Water samples were stored in 60 mL acid-washed LDPE bottles. Water samples were fixed in 2% trace metal grade nitric acid after collection and refrigerated until analysis.

Water samples were analyzed at the University of Texas at Austin, Jackson School of Geosciences at. Samples were analyzed for trace elements (^{88}Sr, ^{137}Ba, ^{55}Mn, ^{24}Mg, ^{40}Ca) using an Agilent 7500ce quadrupole inductively coupled plasma mass spectrometer (ICP-MS) run in solution mode. Prior to analysis, samples were diluted by a factor of 10, 20, or 100x (depending on sample salinity) using 2% nitric acid to obtain less than 500 ppm total dissolved solids. Machine drift was compensated for by spiking selected samples with an internal standard solution. Mean recovery for spiked elements was 99%. Accuracy was calculated within 10% for all elements by using National Institute of Standards and Technology (NIST) 1643e as an external reference standard, diluted 10x. Water samples were analyzed for Sr isotopes using procedures detailed in Banner and Kaufman (1994). Analyses were conducted using a Finnigan-MAT 261 thermal ionization mass spectrometer in static multi-collection mode. Twenty analyses of standard reference material NIST 987 yielded a mean $^{87}Sr/^{86}Sr$ value of 0.71025, $\pm 1 \times 10^{-5}$ (2σ) and within error of the accepted SRM 987 value of 0.71026.

Otolith chemistry

Wild juvenile and adult southern flounder were collected from areas around Port Aransas, Texas, USA from October 2010 through January 2012 (Appendix B). Juvenile fish were collected using seines and an otter trawl. Adult fish were collected using gigging, trawling, hook and line, dip netting, and seining. Immediately after capture, fish were euthanized using a clove oil and ice slurry followed by the severing of the spinal cord. Total length of each individual and sex, if it could be discerned, was recorded. Otoliths were extracted from fish, sectioned, aged, and cleaned according to procedures modified from those detailed by Secor (1992), briefly described here. Extracted otoliths were rinsed of excess tissue and stored dry. Southern flounder otoliths are asymmetrical, and therefore only the left sagittal otolith, which is the larger of the two, was used for microchemistry and ageing. The left otolith from each individual was embedded in EpoxiCure ® Epoxy Resin, mixed with EpoxiCure ® Epoxy Hardener in a 5:1 ratio (Buehler, Lake Bluff, Illinois, USA). After 24 h, otoliths were sectioned in 1 mm sections along the transverse plane using a Buehler Isomet ™ low speed saw with a diamond wafering blade

(Buehler, Lake Bluff, Illinois, USA). Sections were then mounted to a petrographic slide using Crystal Bond ® 509 (Ft. Washington, Pennsylvania, USA). Otoliths were polished to expose the core, first using 30 micron lapping film and then 3 micron lapping film (3M, St. Paul, Minnesota, USA). Once the core was in view, the section was then removed from the petrographic slide and remounted on a new petrographic slide (6 otoliths per slide) for chemical analysis. Prior to analysis, surfaces of sectioned otoliths were cleaned using sonication. Slides containing the otoliths were first dipped in ultrapure water (18.2 MΩ·cm) and then scrubbed for one minute using a soft-bristled toothbrush. The slide was then transferred to a plastic, acid washed beaker, covered with ultrapure water and placed in a sonicating water bath. After two minutes of sonication (50/60 Hz), the slide was removed and triple rinsed with ultrapure water. Slides were then air dried under a class 100 laminar flow hood and sealed in plastic petri dishes for transport.

Otoliths were analyzed for trace element (Sr/Ca and Ba/Ca) and isotope ($^{87}Sr/^{86}Sr$) ratios at the Jackson School of Geosciences at the University of Texas at Austin. Trace elements were quantified using laser ablation ICP-MS on an Agilent 7500ce quadrupole ICPMS coupled to a 193nm New Wave UP-193FX excimer laser system. Samples were loaded into a large-format laser cell that allowed multiple slides to be loaded into the chamber at once, reducing wash-out time and background equilibration. Otolith runs were bracketed by two carbonate certified reference materials, NIST 612 and MACS3. Prior to ablation, analysis tracks across otoliths were pre-ablated using a low-powered laser cleaning pulse, with a spot size of 50 µm, to remove surface contamination. Otoliths were analyzed for ^{137}Ba, ^{55}Mn, ^{24}Mg, ^{88}Sr, and ^{43}Ca. Otoliths were ablated from the distal edge of the core, across the core, and to the edge of the otolith along the sulcal groove. A spot size of 35 µm with a scan speed of 3 µm/s was used.

Elemental counts were converted to element:Ca ratios using the bracketed standard approach described by Rosenthal et al. (1999). Briefly, background elemental intensities were subtracted from all measurements, correction factors for elemental mass bias were calculated and linearly interpolated between adjacent analyses of the MACS3 standard, and finally precision was assessed by replicate measurements of the NIST 612 standard. Estimates of analytical precision (relative standard deviation) across all runs was (n = 38) was 5.5% for Sr/Ca and 10.4% for Ba/Ca. Elemental profiles for each otolith were then filtered using an 11 point mean to improve the signal-to-noise ratio (Sinclair et al. 2012).

Sr isotopes were quantified using a ThermoFinnigan Neptune coupled to a 193nm New Wave UP-193FX excimer laser system. Samples were loaded into a large-format laser cell. Prior to every sample, a gas blank was run to quantify background elemental levels in the chamber. Additionally, prior to analysis a 500 µm transect was laid down adjacent to the transect that was to be analyzed. This additional transect was used for collecting background. Prior to ablation, analysis tracks across otoliths were pre-ablated using a low-powered laser cleaning pulse, with a spot size of 50 µm, to remove surface contamination. Otolith runs were bracketed by a carbonate reference standard (FEBS-1). Otoliths were analyzed for ^{82}Kr, ^{83}Kr, ^{84}Kr, ^{85}Rb, ^{86}Kr, ^{87}Sr, and ^{88}Sr. Otoliths were ablated from the distal edge of the core, across the core, and to the edge of the otolith along the sulcal groove. A spot size of 50 µm with a scan speed of 5 µm/s was used. Concentrations of isotopes obtained were compared to known values of isotopic ratios to calculate the $^{87}Sr/^{86}Sr$ ratio.

Otolith aging

After laser analysis, otoliths were first photographed under a 10x magnification and were then aged using procedures detailed in VanderKooy (2009). Briefly, the first annulus was identified as the first complete opaque band nearest to the core (VanderKooy 2009). Opaque bands were counted to determine the age of the individual. In the absence of an opaque band, the individual was judged to be less than 1 year old. Image analysis software ImageJ (http://rsb.info.nih.gov/ij/) was then used to measure otolith accretion. Otolith accretion for the first year was measured from the center of the otolith core, to the distal edge of the first annulus along the sulcal groove. When possible, the accretion rate was measured alongside the laser transect. Based on a birth date of January 1 (Wenner et al. 1990), fish were classified into year classes based on the age determined.

Data analysis

To identify major shifts in elemental profiles across each otolith, profiles were processed using a global zoning algorithm. This algorithm, developed by Hedger et al. (2009), uses a recursive procedure to divide the otolith transect into a series of zones with distinct mean chemical compositions, therefore allowing for the quantitative distinction of significantly different chemical zones. By definition, this zoning algorithm removes high frequency variability much like a smoothing procedure, and therefore provides a more conservative measurement of lifetime variation in the chemical proxy (Walther et al. 2011).

The calculation of a partition coefficient allowed for individual life history profiles to be examined and categorized into different low salinity habitat use categories based on the proportion of the life history transect that was on either side of thresholds that defined residence in low salinity habitat. A partition coefficient describes the proportion of a dissolved constituent (e.g. Ba/Ca) that is ultimately incorporated into the otolith (Morse and Bender 1990). The partition coefficient (D_{Me}) is calculated using the formula:

$$D_{Me} = \frac{(Me:Ca)_{otolith}}{(Me:Ca)_{water}}$$

where *Me* is the element of interest. For this project, D_{Ba} was calculated using analyses of otoliths from fish that were reared in fully marine water and analyses of Ba/Ca ratios in fully marine water (see Chapter 2 for details). Applying the partition coefficient to the elemental composition of local tributaries, threshold Ba/Ca values in otoliths were calculated that indicate movement into low salinity (salinity $\leq$5) and possibly fresh water. Freshwater Ba/Ca values were found to be highly variable among tributaries in the region, and therefore thresholds for movement into low salinity habitat were used that reflected the mean freshwater Ba/Ca value as well as low (minus one standard deviation) and high (plus one standard deviation) thresholds. All three of these thresholds were used to assess individual life history profiles.

Threshold values were used to categorize each data point along the life history profile of each otolith as residence in low salinity habitat or estuarine/marine habitat to determine the percentage of its lifetime that an individual spent in low salinity habitat. As previously discussed, otolith accretion for the first year of life was measured, thus allowing for each profile to be partitioned into the first year and post year 1.The proportion of time that an individual spent above the low salinity habitat threshold was compared in three different ways: 1.) for the entire life history profile of each fish, 2.) for the first year of life (up to the first annulus, or the otolith edge for age 0 fish), and 3.) after the first annulus (for fish ages $\geq$ 1.) Individuals were grouped into four categories of low salinity habitat usage (0 – 25%, 26 – 50%, 51 – 75%, 76 – 100%), where the percent of low salinity habitat usage represents the percent of their life history profile that was above the threshold. For each of these categories, the proportion of low salinity residence seen throughout the life profile was determined using the three Ba/Ca thresholds (low, mean, and high). Additionally, profiles were categorized using the mean threshold after processing with the global zoning algorithm.

Results

Water chemistry

For this analysis, the Ba/Ca ratio of all sites that had a salinity of less than 5 were included in the calculation of the freshwater endmember, resulting in a mean freshwater endmember value of 568.10 $\pm$ 359.83 μmol/mol Ba/Ca (1 SD). In the water sampling survey, not all sites samples were above tidal influence and therefore, the salinity at some sites changed between years, affecting the Ba/Ca ratio. Therefore, this value does not represent that of a pure freshwater endmember. Removing the sample sites that were below tidal influence, the mean freshwater endmember value is 414.64 $\pm$ 220.22 μmol/mol Ba/Ca (1 SD). Applying this mean freshwater endmember value to the partition coefficient would yield a mean freshwater threshold value of 16 μmol/mol Ba/Ca. However, the mean freshwater endmember value of 568.10 $\pm$ 359.83, and therefore the mean freshwater threshold value of 20 μmol/mol was used to give a more conservative estimate of low salinity habitat use. Additionally, during the time of year that juvenile southern flounder would be using low salinity habitat, it is possible that due to low flow rates and drought conditions, these juvenile southern flounder would not be able to be exposed to the chemical environment of the pure freshwater endmember. Therefore, the use of the endmember value of 568.10 $\pm$ 359.83 µmol/mol Ba/Ca, while it does not represent a true endmember values, is representative of the conditions that juvenile southern flounder would be experiencing if they were residing in low salinity water. Additionally, the value that is used to represent the freshwater endmember value falls within the range of freshwater Ba/Ca values seen in the literature (200 – 1600 µmol/mol Ba/Ca) (Jessop et al. 2012, Miller et al. 2010).

Interannual variability was observed within tributaries across both years, for both Ba/Ca (Figure 1.2) and Sr/Ca (Figure 1.3). The variability within tributaries between years can most likely be attributed to the differences in salinity at the same site across years (Appendix A). Although there was variability in the Ba/Ca concentrations within tributaries between years, a two-tailed t-test indicated that the differences between years were not significant for the Nueces River (p=0.22), the Guadalupe River (p=0.14), Oso Creek (p=0.16), and the San Antonio River (p=0.39). There was a significant difference in the Ba/Ca concentrations between years for the Aransas River (p=0.03).For the Sr/Ca concentrations, there were no significant differences detected in the concentrations between years ($p > 0.05$).

Both Ba/Ca and Sr/Ca concentrations displayed the expected patterns across the salinity gradient (Figures 1.2 and 1.3). In general, as salinity increases Ba/Ca decreases, while Sr/Ca demonstrates the reverse pattern. Overall, the mean freshwater Ba/Ca value was 568.10 $\pm$ 359.83 µmol/mol (1 SD). The mean freshwater signal for each tributary shows that there is a wide degree of variation in the Ba/Ca values in rivers in the Coastal Bend of Texas (Figure 1.4). Oso Creek exhibits the lowest mean Ba/Ca freshwater signal with (212.19 $\pm$ 101.29 µmol/mol), while Mission River has the highest (1116.93 $\pm$ 18.90 µmol/mol). The mean Sr/Ca freshwater signal for each tributary shows that there is less variation in Sr/Ca values among tributaries as compared to Ba/Ca values (Figure 1.5).

Consistent with the ratios of Ba/Ca, Sr/Ca, and $^{87}Sr/^{86}Sr$ in local rivers, Ba/Ca was found to be the most useful tool for salinity reconstruction based on the shape of the mixing curves across salinity gradients in this system. Although the global zoning algorithm did quantitatively identify significant zone differences in otolith Sr/Ca (Figure 1.6), water chemistry patterns prevented the meaningful interpretation of these results given that there was not a significant amount of difference between the fresh and marine endmembers (Figure 1.7). In contrast, Ba/Ca exhibited a mixing curve that would make it an ideal candidate for salinity reconstruction (Figure 1.8). For water $^{87}Sr/^{86}Sr$ ratios, with average freshwater $^{87}Sr/^{86}Sr$ ratios (0.7082) being only marginally different than the globally homogeneous marine value (0.7092), also indicating that $^{87}Sr/^{86}Sr$ is not a useful tool for salinity reconstruction in this system (Figure 1.9). Using $^{87}Sr/^{86}Sr$ ratios was further complicated by the degree of analytical noise in measurements of otolith $^{87}Sr/^{86}Sr$. The mean $^{87}Sr/^{86}Sr$ value seen in the otoliths examined was 0.709 ± 0.0004 (Figure 1.10), which was within two standard deviations of both the global mean $^{87}Sr/^{86}Sr$ value (0.7092) and the average freshwater $^{87}Sr/^{86}Sr$ value in this region (0.7082). This means that although the difference between fresh and marine $^{87}Sr/^{86}Sr$ endmembers was observed in the 4th decimal place, high instrumental noise in otolith $^{87}Sr/^{86}Sr$ measurements also occurred in the 4th decimal place. Any movements across salinity gradients recorded by this proxy would therefore be obscured by analytical error, rendering this proxy not useful for this project.

Otolith chemistry

For the reasons given above, all remaining analyses on otolith chemistry focused on Ba/Ca values exclusively. As detailed in Chapter 2, the mean marine otolith Ba/Ca value for captive fish was calculated to be 2.86 µmol/mol. Using the partition coefficient formula, and a mean experimental and wild marine water Ba/Ca value of 71.70 µmol/mol, the partition coefficient was calculated to be 0.04 $\pm$ 0.006 (1 SD). This partition coefficient was applied to the mean freshwater Ba/Ca value for local rivers (568.10 $\pm$ 359.83 µmol/mol), to calculate a mean otolith low salinity threshold value of 20 µmol/mol Ba/Ca. Therefore, any otolith value over 20 µmol/mol Ba/Ca was interpreted as residence in low salinity habitat. Using the standard deviation around the mean freshwater Ba/Ca value, both a high otolith threshold of 32 µmol/mol and a low otolith threshold of 8 µmol/mol Ba/Ca were calculated. These three thresholds (low, mean and high) were used to determine the proportion of low salinity habitat use exhibited in each individual life history.

Otolith Ba/Ca values revealed that there was a high degree of variability in habitat use patterns among individuals. Low salinity habitat use was categorized into four groups (0 – 25%, 26 – 50%, 51 – 75%, 76 – 100%). When the complete life history profile of each individual was evaluated using the mean threshold, 80% of individuals spent 0 – 25% of their lifetime in low salinity habitat (Table 1.1). While the majority of individuals exhibited little or no low salinity habitat use, 8% of individuals used low salinity habitat for 26 – 50% of their lives, 7% for 51 – 75% of their lives, and 4% for 76 – 100% of their lives (Table 1.1). The proportion of individuals assigned to each category of habitat usage varied depending on the threshold value used (Figure 1.11). The low threshold categorized the smallest proportion of fish into the 0 – 25% usage category with 25%, while the high cutoff categorized the highest proportion of fish to this category (91%, Table 1.1). The zoned values for each category were similar to the mean values, which is not surprising given that the mean threshold of 20 μmol/mol was used to evaluate the zoned values. When the 0 – 25% low salinity habitat usage category is examined more closely, it is revealed that, when evaluated using the mean threshold value, 41% of individuals never used low salinity habitat throughout their entire lives (Table 1.1). This also indicates that 59% of individuals examined used low salinity habitat at some point during their life. The mean percent time spent in low salinity (evaluated using the mean threshold value) across all of the individuals was 15%.

When life history profiles of each individual are examined using the part of the otolith transect that represented the first year of life, the proportion of individuals grouped into each low salinity use category remained similar to the proportions seen when examining the entire transect (Table 1.2). Using the mean threshold, the highest proportion of individuals (78%) used low salinity habitat for 0 – 25% of their lives. Again, the low threshold categorized the smallest proportion of fish into the 0 – 25% range (34%), while the high threshold categorized the largest proportion of fish into the 0 – 25 % range (88%). When the 0 – 25% category is further evaluated, 55% of individuals do not enter low salinity habitat during the first year of life, while 45% of individuals do utilize low salinity habitat during the first year of life. The zoned data finds that an even higher proportion (65%) of individuals do not use low salinity habitat during the first year. Again, the percent of time that an individual spent in low salinity habitat varies with the threshold used (Figure 1.12). The mean percent time spent in low salinity habitat during the first year (evaluated using the mean threshold value) was 15%.

When the life history profile of each individual is examined using only the part of the otolith transect that represents the post-year one movements, 51% of individuals did not enter low salinity habitat after the first year of life (Table 1.3). Again, the low threshold categorized the smallest proportion of fish into the 0 – 25% range (22%), while the high threshold categorized the largest proportion of fish into the 0 – 25 % range (89%, Figure 1.13). The mean percent time spent in low salinity habitat after the first year (evaluated using the mean threshold value) was 20%.

When the proportion of individuals in each of these low salinity habitat usage categories is examined by age class (using the complete life history transect), age 0 exhibits the highest proportion of individuals in the 0 – 25% usage range (Table 1.4). However, this result could be due to these individuals not yet having the opportunity to use low salinity habitat or skewed proportions given the small sample size for this age class (n = 19). Age 1 individuals exhibited the second highest proportion of individuals in the 0 – 25% usage category (82%). Age 3 had the highest proportion of individuals grouped into the 76 – 100% low salinity habitat usage category (33%), although the sample size of age 3 individuals was small (n = 6). Age 2 had the second highest proportion of individuals grouped into the 76 – 100% low salinity habitat usage category, with 13%. Age 0 had the highest proportion of individuals that never used low salinity habitat (67%), while age 1 had the second (57%). Age 3 had the highest proportion of individuals that used low salinity habitat (83%), followed by age 2 (59%).

When the life history profiles are examined by year class, the 2011 year class had the highest proportion of individuals that fall into the 0 – 25% usage category (93%, Table 1.5). While not all age 0 individuals examined were of the 2011 year class, this result is not surprising given that age 0 was found to have the highest proportion of individuals in the 0 – 25% low salinity habitat usage category when low salinity habitat usage was compared among age classes. When the percentage of time spent in low salinity habitat in a year class is compared with the flow rates of local rivers (Figure 1.14), the years with comparatively lower flow rates do not always match up with the year classes with the lowest proportion of low salinity habitat use. For example, 2008 was a year with relatively low flow rates, yet the 2008 year class had the lowest percentage of fish grouped into the 0 – 25% category (48%). Flows were relatively high in 2010, yet the 2010 year class had a relatively high proportion of individuals grouped into the 0 – 25% low salinity habitat usage category (85%). Flow rates were also relatively high in 2007, and the 2007 year class represents the lowest proportion of fish that fell in the 0 – 25% category (0%), but the sample size was very small for this year class.

Several life history profiles representative of the elemental patterns seen in individual otoliths are presented here. Life history profiles of individuals not shown here can be found in Appendix B. As previously discussed, when evaluated using the mean threshold (and the data from the entire otolith transect), 41% of individuals never ventured into low salinity habitat. These individuals exhibited low Ba/Ca values throughout their lives (Figure 1.15). Neither the smoothed data values nor the zoned values went above the mean threshold. While the remaining 59% of individuals did venture into low salinity habitat, the timing, frequency, and duration of low salinity habitat use differed among individuals. Some individuals spent their early life stages in the marine environment, made an excursion into low salinity habitat, and then moved back into marine habitat for the remainder of their lives (Figure 1.16). The excursion into the low salinity environment is indicated by the smoothed data points being above the mean threshold, but the zoning algorithm also identifies this movement as a quantitatively different chemical environment experienced by the individual. After spending their early life stages in the marine environment, other individuals made an excursion into low salinity habitat and remained in the low salinity environment for the remainder of their lives (Figure 1.17). Some fish show variability in using the marine and estuarine habitats, while never entering low salinity habitat (Figures 1.18 and 1.19). Additionally, there were some unexpected patterns seen in the Ba/Ca values. Several individuals displayed high Ba/Ca otolith concentrations very early in life (Figures 1.20 and 1.21), which is unexpected given what is known about southern flounder life history and the survival of southern flounder eggs and larvae in low salinities. The movement patterns between the fresh, estuarine, and marine environments were quantitatively identified by the global zoning algorithm.

In comparing the percent of time that an individual spent in low salinity habitat during the first year of life and otolith accretion during the first year (as a proxy for growth), a linear regression indicated that for fish aged 0, there was a significant relationship between the percent time spent in low salinity habitat and accretion, when the analysis was done using the 20 μmol/mol threshold ($p = 0.02$, Figure 1.22). However, this relationship did not hold true across all age classes. For age classes 1, 2, and 3, there was no significant relationship between the accretion rate during the first year and the percent of time spent in low salinity habitat ($p > 0.05$, Figure 1.23, 1.24, 1.25, 1.26). There was no linear regression calculated for age class 4 because there were only two individuals that were part of this age class. For all age classes, accretion varied widely.

Discussion

This study used otolith Ba/Ca patterns to demonstrate that a significant proportion of juvenile southern flounder use low salinity habitat and confirmed Ba/Ca to be a reliable and informative tracer in reconstructing the salinity history of juvenile southern flounder. Although Sr/Ca and $^{87}Sr/^{86}Sr$ are frequently used in other systems to reconstruct the movement patterns of fish across a salinity gradient, the geology of the Texas coastal plain with its dominant marine-derived carbonate formations prevents these tracers from being useful in this system. In comparison with other systems, the south Texas tributaries have much higher Sr/Ca values in fresh water, so that differences between fresh and marine endmembers are relatively small and making Sr/Ca a less reliable tool for salinity reconstruction. For example, in the San Francisco Estuary, where otolith Sr/Ca has previously been used to reconstruct salinity history, the difference between the Sr/Ca freshwater and marine endmembers is approximately 8 mmol/mol Sr/Ca (Phillis et al. 2010), which is double the difference in endmembers found in this study of approximately 4.5 mmol/mol Sr/Ca.

Although there was variation in Ba/Ca concentrations among tributaries between years, the Ba/Ca freshwater values are significantly different enough from the Ba/Ca marine values to allow for the identification of freshwater and low salinity movements. The variability in Ba/Ca concentrations seen can be attributed to the differences in salinities experienced at the same sites in different years, something that likely reflects the different flow rates of 2010 and 2011. However, the large difference between the concentrations of Ba/Ca in the freshwater and the marine endmember, as well as varying predictably based on salinity, allows Ba/Ca to be an informative tracer, even with these interannual variations. The variability of Ba/Ca values in the freshwater endmembers was taken into account when interpreting the life history profiles by creating low, mean, and high thresholds. Although the threshold used could alter the low salinity categorization of individuals, the mean threshold appears to be a likely and reasonable threshold for evaluating the movement patterns of juvenile southern flounder. While it is important to consider the elemental variability in freshwater sources, the mean freshwater endmember value is similar to the Ba/Ca concentrations of the major tributaries to the local bay systems (Guadalupe, San Antonio, and Nueces). The tributaries where Ba/Ca values were comparatively higher (Mission River) or lower (Oso Creek), do not contribute as much flow to the local bay systems. Therefore, it is reasonable to assume that any otolith Ba/Ca values above 20 μmol/mol are indicative of low salinity habitat use.

Although the movement patterns of individuals could be grouped into general categories, there was a high degree of individual variation in the habitat use patterns of juvenile southern flounder. A significant finding of this study is that the majority (59%) of southern flounder examined use low salinity habitat at some time during their lives, and 45% use low salinity habitat at some point during their first year of life. Juvenile and adult southern flounder have previously been collected in fresh water and low salinities throughout its range but this study quantifies the proportion of individuals in the population that use low salinity habitat throughout their lives and identifies the percent of an individual's life history that is spent in the low salinity environment. Recent research in the Mobile-Tensaw Delta (AL) identified that juvenile southern flounder extensively use freshwater habitat (Lowe et al. 2009). All of the juveniles examined had otolith life history profiles that were indicative of freshwater habitat use. However, the work done by Lowe et al. (2009) only examined individuals captured in fresh water, therefore precluding the ability to determine whether individuals that did not move into low salinity waters were present. In contrast, the present study also confirms the importance of the marine and estuarine habitat to juvenile southern flounder, with 41% of individuals never entering low salinity habitat. It has been previously established that southern flounder is a euryhaline species, but this study identifies that there are two distinct contingents in the southern flounder population: one that uses low salinity habitat and one that does not. However, given the variability seen in the movements of southern flounder, this is a rather coarse distinction. Although individuals can be categorized into those that use low salinity habitat and those that do not, habitat use patterns were so variable among individuals that it is more realistic to describe the habitat use patterns of southern flounder as a gradient. Within year classes and age classes there are some individuals that never enter low salinity habitat, some who spend nearly their whole lives in low salinity habitat, and individuals everywhere in between. However, the mean percent time spent in low salinity (evaluated using the complete life history profile and the mean threshold value) was 15%, indicating that low salinity habitat usage is strongly skewed toward the lower end. However, there are some individuals (4%) that spent 75 – 100% of their life in low salinity. The identification of contingents within a population and the habitat that they use is critical to conservation plans, therefore an understanding of the low salinity habitat use patterns of juvenile southern flounder and the proportion of their lives spent in this habitat can help to develop plans to conserve this important species.

Interestingly, when the otolith transect of all individuals are examined using only the part of the otolith that represents the post-year one movements, the proportion of individuals grouped into the four categories of low salinity habitat usage remains similar to the results seen when only the year one data is examined. This indicates that, for the most part, that if an individual chooses to use low salinity habitat early in its life history, that individual is more likely to use low salinity habitat later in life (post yr-1) than an individual that did not enter low salinity habitat during its first year. This indicates that low salinity habitat could be vital to not only juvenile southern flounder, but adult southern flounder as well. This study specifically focuses on quantifying the amount of time that juvenile southern flounder spend in low salinity habitat. However, as can be seen from the individual life history profiles, there is considerable variation among individuals in their movements that occur below the threshold. For example, the individual depicted in Figure 1.15 spends its entire life below 10 μmol/mol of Ba/Ca, indicating that this fish stayed in the marine environment for its entire life. However, the individuals shown in Figures 1.18 and 1.19 move between the marine and estuarine environments throughout their life until capture in the marine environment.

One of the limitations of otolith microchemistry in reconstructing habitat use patterns is that it is difficult to distinguish if the individual is making a behavioral choice to seek out a different environment or if the chemical environment around the individual is changing. I had hypothesized that the years with the lowest flow rates would correspond to the years where individuals used the lowest proportion of low salinity habitat use and the years with higher flow rates would correspond to the years where individuals used a higher proportion of low salinity habitat use. An interesting finding is that the year class with the highest proportion of low salinity habitat usage did not match up to the year with the highest flow rates. For example, 2010 year class had a higher proportion of individuals that never used low salinity habitat (62%), as compared to the 2011 year class (53%), although there were higher flow rates in 2010. This is an indication that when a juvenile southern flounder inhabits low salinity habitat, it is most likely making a behavioral choice, rather than the chemical environment around the individual changing. However, our sampling of year classes was not even and some year classes were represented by small sample sizes, meaning that definitive conclusions about the diversity of habitat residence patterns in some year classes cannot yet be made. Further investigation into the behavioral choices of southern flounder juveniles using low salinity habitat is warranted.

Another caveat of using otoliths to reconstruct migration patterns is that there can be a significant time lag between when a fish is exposed to an element in the water and when that element appears, at equilibrium, in the otolith (discussed further in Chapter 2). For example, in Figure 1.18, there seemed to be a cyclic pattern in the individual's movements. Although the otolith Ba/Ca values do not indicate that the fish ever went back below the threshold, it is possible that the fish made the movement into and out of the marine environment so rapidly that there was never enough time for the otolith to equilibrate with, or even reflect, the marine signal. On the other hand, it is possible that some of the fish that exhibit Ba/Ca values that are near the threshold value did venture into low salinity habitat but not for a long enough time that it was recorded in the otolith. Additionally, all of the southern flounder used in this study were caught in the marine environment, but Ba/Ca values on the edge of the otolith sometimes were found to be above the mean threshold. The effect of a time lag could also explain some of the unexpected patterns that were seen in some of the life history profiles of individuals examined in this study. Similar to the findings of Lowe et al. 2010, some individuals that exhibited high otolith Ba/Ca values in the core of the otolith. Typically, this would be interpreted as fish being hatched in low salinity habitat. However, previous studies have established that southern flounder eggs and larvae exhibit low survival in fresh water (Smith et al. 1999). Therefore, these high Ba/Ca values very early in life likely indicates that these individuals moved into fresh or low salinity water so quickly after they were hatched that no marine signal is detectable in the otolith, or that the otolith was sectioned such that the earliest marine material was not exposed for ablation.

A third limitation of using otoliths is that the temporal resolution of analyses changes with fish age. The most rapid period of somatic and otolith growth is during the first year of life, meaning that there is much more material in the otolith representing the first year of life as compared to subsequent years. After the first year of life, when otolith growth slows, accretion is much lower. Each laser spot is therefore integrating across more time as a fish grows older meaning that when a life history profile is examined, it is important to keep in mind that the potential to detect rapid movements of an individual becomes diminished with age. Therefore, only longer-term sustained movements can be examined in the life history profile of otoliths when fish are greater than 1 year old. This indicates that estimates of the time that an individual spent in low salinity habitat that are made in this study are potentially conservative, as the rapid movements across the salinity gradient may not appear in the otolith.

Now that the use of low salinity habitat by juvenile southern flounder in south Texas has been identified and quantified, this information can be considered when managing southern flounder populations and also environmental flows. In addition to establishing the presence of a contingent of juvenile southern flounder that use low salinity habitat, this study also has highlighted the high degree of variability in regards to habitat use among individuals. Although the importance of freshwater inflows to estuaries has been well established, this work demonstrates the importance of freshwater inflows to the southern flounder population in Texas.

A high proportion of the southern flounder population chooses to utilize low salinity habitat during the juvenile life stage, and maintaining this habitat could be critical to the maintenance of the southern flounder populations in Texas. Although the dominant life history strategy does not include measureable time spent in low salinity waters, the maintenance of low salinity habitat use in the southern flounder population is critical. As mentioned previously, contingents can have important impacts on the population dynamics of a species, including maintaining population stability and the mitigation of detrimental environmental impacts. The importance of freshwater inflows and how freshwater inflows impact the distribution and abundance of other fish species has already been documented (Longley 1994), but this study shows how freshwater inflows have the potential to impact southern flounder population dynamics. The high degree of variation in the proportion of individuals that use low salinity habitat between year classes indicates that this population has the ability to rapidly adapt to changing conditions, even during the juvenile phase. Now that the presence of different contingents has been established in the population, further work can be conducted to better understand the mechanism that drives the use of low salinity habitat. Previous research has identified that differential growth rates, sex, and resource availability all play a role in the definition of contingents in other marine species (Kerr et al. 2009). This study investigated the relationship between growth, as measured by otolith accretion rate in the first year, and the percent of time that an individual spent in low salinity habitat. Only for fish aged 0 was there a weak relationship between the percent of time spent in low salinity habitat and otolith accretion. However, otolith accretion as measured over the entire first year is a coarse measure of growth. It is possible that growth is still critical in determining the habitat use patterns of a fish, but only the growth rate at a particular time is critical, rather than the growth averaged across the first year of life.

This study has demonstrated that there are two distinct contingents that form during the juvenile life stage of southern flounder: one that uses low salinity habitat and one that does not. Within the contingent that does use low salinity habitat, there is a high degree of variability in the amount of time spent in low salinity habitat, as well as the timing, frequency, and duration of movements into low salinity waters. To better understand the contingents within the southern flounder population and what drives them, it is critical to examine the patterns behind the formation of contingents and the mechanisms driving low salinity habitat use in southern flounder populations. Although fishing pressure currently represents the largest threat to the sustainability of the southern flounder population, an understanding of the importance of low salinity habitat to juvenile southern flounder populations and migratory behaviors during the juvenile life stage is important in understanding southern flounder population dynamics. With decreasing future precipitation rates and increasing anthropogenic alteration of freshwater inflows, and a southern flounder population that exhibits a high number of individuals that use low salinity habitat, it is important to consider how the alteration of freshwater inflows will impact the southern flounder population.

Chapter 1 Tables

Threshold Value	% Time in Low Salinity (≤5)			
	0 - 25	*26 - 50*	*51 - 75*	*76 - 100*
Low	25% (65)	23% (60)	20% (51)	32% (83)
Mean	80% (207)	8% (22)	7% (19)	4% (11)
High	91% (235)	6% (15)	3% (7)	1% (2)
Zoned	80% (207)	10% (25)	5% (13)	5% (14)

A.

Threshold Value	% Time in Low Salinity (≤5)					
	0	*1 - 5*	*6 – 10*	*11 – 15*	*16 - 20*	*21 - 25*
Low	5% (13)	3% (7)	7% (17)	3% (8)	4% (10)	4% (10)
Mean	41% (106)	12% (31)	9% (23)	6% (16)	8% (22)	3% (9)
High	67% (173)	12% (30)	3% (9)	3% (9)	3% (9)	2% (9)
Zoned	52% (135)	1% (2)	5% (14)	9% (24)	7% (17)	6% (15)

B.

Table 1.1. A. The proportion of individuals grouped into four categories of low salinity habitat usage. The number of individuals in a given category is listed in parentheses. B. The proportion of individuals grouped into the 0 – 25% category. The number of individuals in a given category is listed in parentheses.

Threshold Value	**% Time in Low Salinity ($\leq$5)**			
	0 - 25	*26 - 50*	*51 - 75*	*76 - 100*
Low	34% (88)	20% (53)	15% (39)	31% (79)
Mean	78% (203)	9% (24)	7% (19)	5% (13)
High	88% (228)	8% (20)	3% (8)	1% (1)
Zoned	78% (203)	10% (27)	6% (15)	5% (14)

A.

Threshold Value	**% Time in Low Salinity ($\leq$5)**					
	0	*1 - 5*	*6 - 10*	*11 - 15*	*16 - 20*	*21 - 25*
Low	11% (28)	3% (8)	7% (17)	4% (11)	3% (7)	7% (17)
Mean	55% (142)	6% (16)	6% (15)	7% (17)	3% (9)	2% (4)
High	76% (196)	5% (13)	3% (8)	2% (4)	2% (4)	1% (3)
Zoned	65% (168)	0% (0)	2% (6)	3% (9)	5% (12)	3% (8)

B.

Table 1.2. A. The proportion of individuals grouped into four categories of low salinity habitat usage. The data shown here is for the first year of life. The number of individuals in a given category is listed in parentheses. B. The proportion of individuals grouped into the 0 – 25% category. The number of individuals in a given category is listed in parentheses.

A.

Threshold Value	% Time in Low Salinity (≤5)			
	0 - 25	*26 - 50*	*51 - 75*	*76 - 100*
Low	22% (54)	14% (35)	13% (32)	50% (123)
Mean	70% (170)	14% (34)	8% (19)	9% (21)
High	89% (218)	7% (17)	2% (6)	1% (3)
Zoned	69% (168)	12% (29)	9% (22)	10% (25)

B.

Threshold Value	% Time in Low Salinity (≤5)					
	0	*1 - 5*	*6 - 10*	*11 - 15*	*16 - 20*	*21 - 25*
Low	7% (18)	3% (8)	4% (9)	2% (5)	3% (8)	2% (6)
Mean	51% (125)	5% (11)	7% (16)	3% (8)	2% (5)	2% (5)
High	77% (187)	4% (10)	3% (7)	2% (5)	1% (3)	2% (6)
Zoned	58% (142)	2% (6)	2% (4)	2% (5)	2% (5)	2% (6)

Table 1.3. A. The proportion of individuals grouped into four categories of low salinity habitat usage. The data shown here is post-year 1, for individuals older than 1. The number of individuals in a given category is listed in parentheses. B. The proportion of individuals grouped into the 0 – 25% category. The number of individuals in a given category is listed in parentheses.

Age Class	% Time in Low Salinity (≤5)			
	0 - 25	*26 - 50*	*51 - 75*	*76 - 100*
Age 0	90% (19)	10% (2)	0% (0)	0% (0)
Age 1	82% (163)	9% (17)	6% (12)	4% (8)
Age 2	59% (19)	13% (4)	16% (5)	13% (4)
Age 3	33% (2)	0% (0)	33% (2)	33% (2)
Age 4	50% (1)	50% (1)	0% (0)	0% (0)

A.

Age Class	% Time in Low Salinity (≤5)					
	0	*1 - 5*	*6 -10*	*11 -15*	*16 - 20*	*21 - 25*
Age 0	67% (14)	14% (3)	5% (1)	5% (1)	0%	0%
Age 1	57% (114)	5% (10)	7% (13)	7% (14)	4% (8)	2% (4)
Age 2	41% (13)	6% (2)	3% (1)	9% (3)	0% (0)	0% (0)
Age 3	17% (1)	17% (1)	0% (0)	0% (0)	0% (0)	0% (0)
Age 4	0% (0)	0% (0)	0% (0)	0% (0)	50% (1)	0% (0)

B.

Table 1.4. A. The proportion of individuals grouped into four categories of low salinity habitat usage by age class using the complete life history profile. The number of individuals in a given category is listed in parentheses. B. The proportion of individuals grouped into the 0 – 25% category. The number of individuals in a given category is listed in parentheses.

Year Class	% Time in Low Salinity (≤5)			
	0 - 25	*26 - 50*	*51 - 75*	*76 - 100*
2006	50% (1)	50% (1)	0% (0)	0% (0)
2007	0% (0)	0% (0)	50% (1)	50% (1)
2008	48% (10)	14% (3)	19% (4)	19% (4)
2009	56% (14)	16% (4)	20% (5)	8% (2)
2010	85% (141)	8% (14)	4% (6)	2% (4)
2011	93% (14)	7% (1)	0% (0)	0% (0)

A.

Year Class	% Time in Low Salinity					
	0	*1 - 5*	*6 -10*	*11 -15*	*16 - 20*	*21-25*
2006	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	50% (1)
2007	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
2008	29% (6)	10% (2)	5% (1)	5% (1)	0% (0)	0% (0)
2009	36% (9)	8% (2)	4% (1)	8% (2)	0% (0)	0% (0)
2010	62% (103)	5% (8)	6% (10)	6% (10)	4% (6)	2% (4)
2011	53% (8)	20% (3)	13% (2)	7% (1)	0% (0)	0% (0)

B.

Table 1.5. A. The proportion of individuals grouped into four categories of low salinity habitat usage by year class using the complete life history profile. The number of individuals in a given category is listed in parentheses. B. The proportion of individuals grouped into the 0 – 25% category. The number of individuals in a given category is listed in parentheses.

Chapter 1 Figures

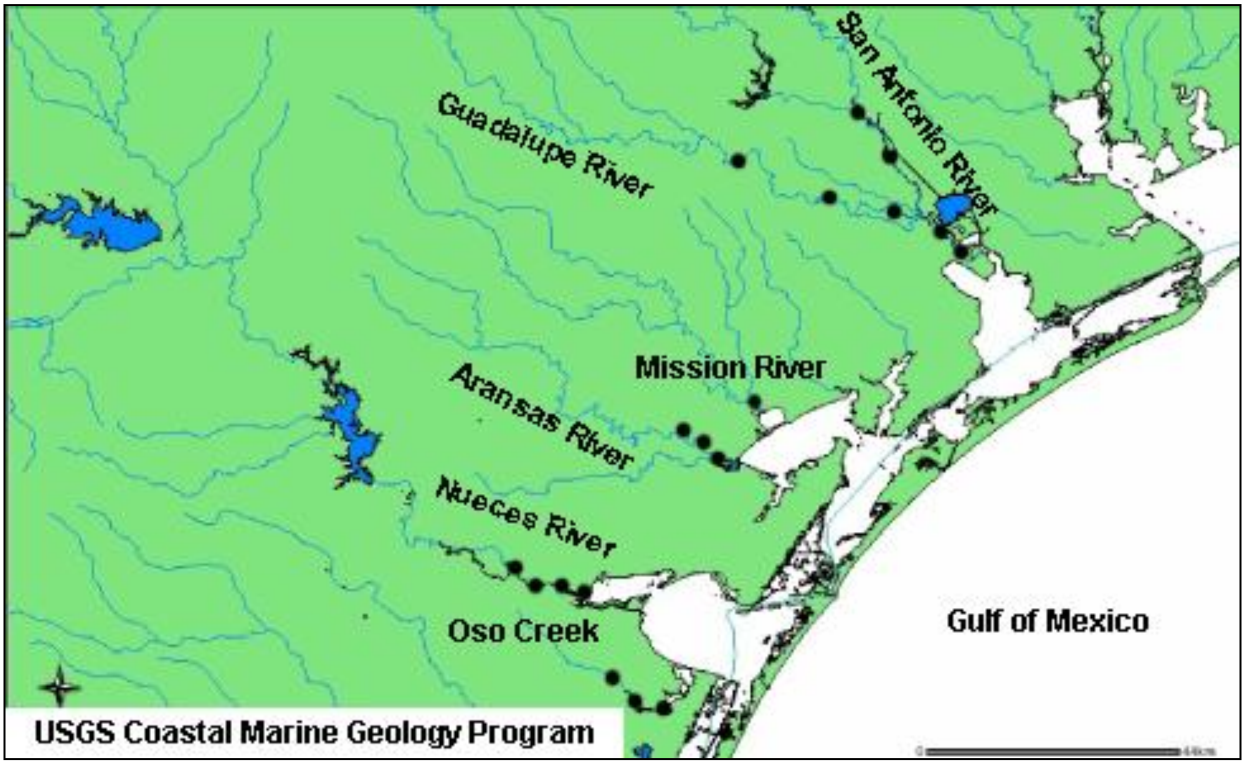


Figure 1.1. A map of the major tributaries to the south Texas Gulf Coast that were sampled in this study. Black dots indicate the sampling sites at each river. Sites on the Mission River are represented by one black dot because they were very close together due to the short reach of the river. In the following figures, the abbreviations for the following tributaries are as follows: Nueces River (NR), Guadalupe River (Guad), Oso Creek (Oso), Aransas River (AR), San Antonio River (SR), and Mission River (MR). Map adapted from the USGS Coastal Marine Geology Program.

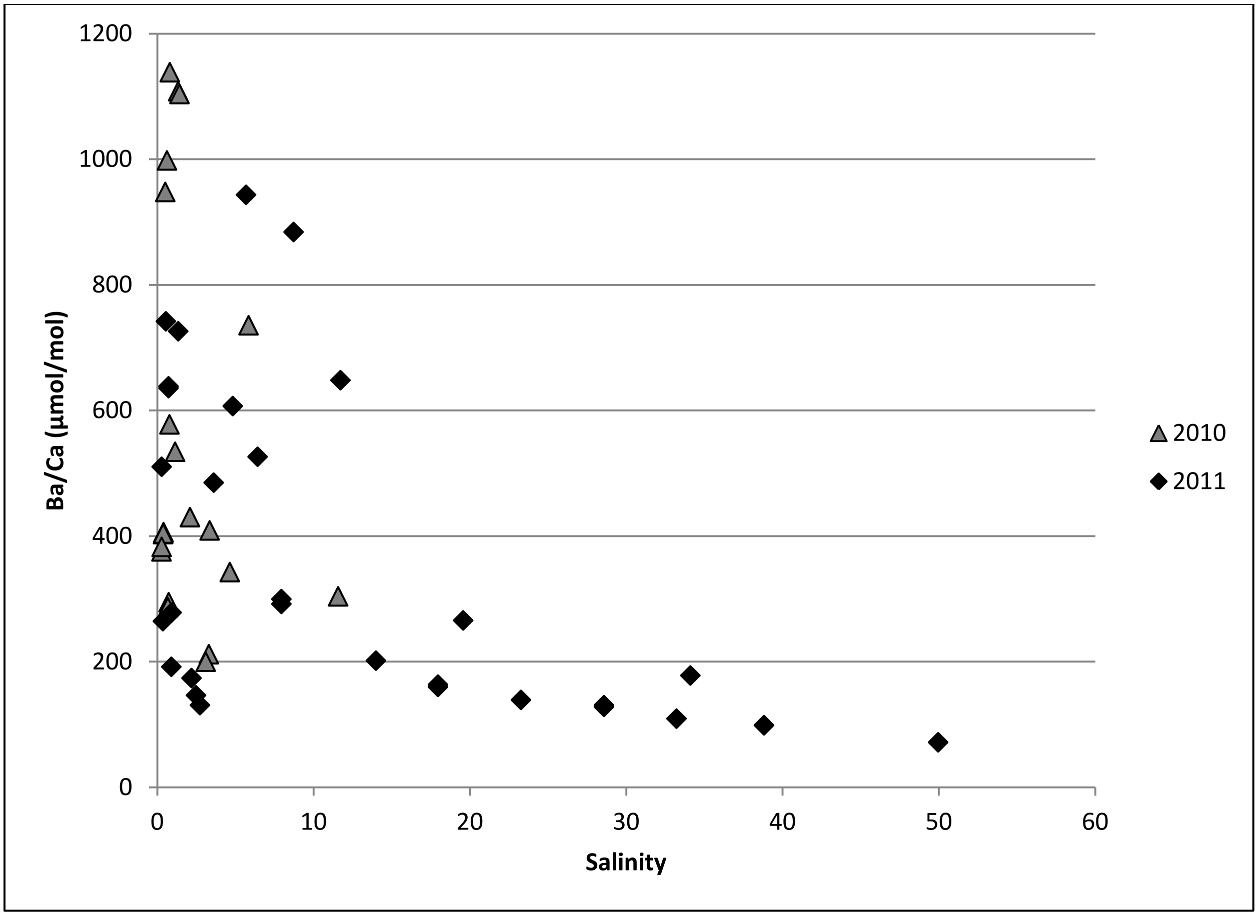


Figure 1.2. Ba/Ca from all sample sites in 2010 and 2011 plotted against salinity. This graph shows the wide variation in Ba/Ca at freshwater sites in south Texas rivers and how the Ba/Ca concentration varies predictably with salinity.

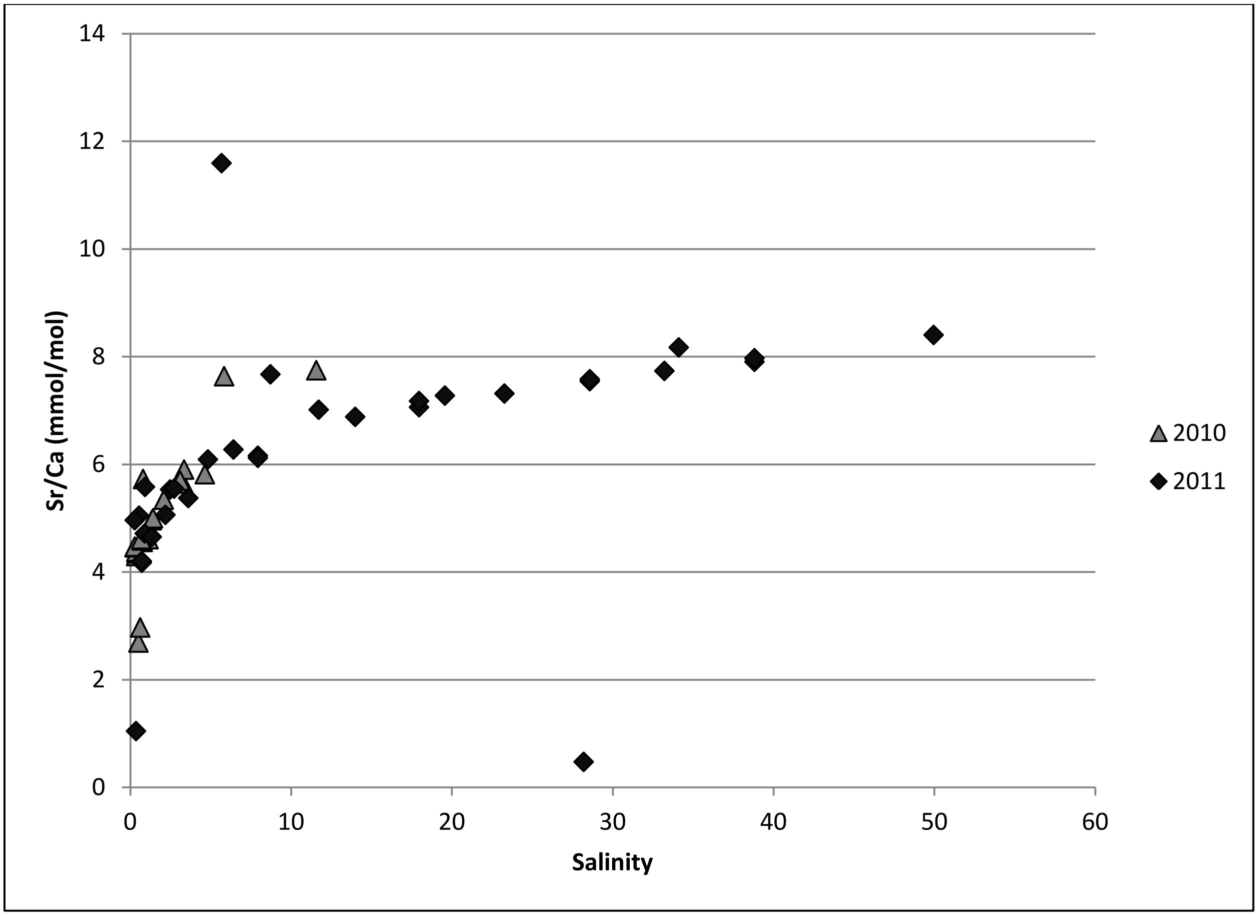


Figure 1.3. Sr/Ca from all sample sites in 2010 and 2011 plotted against salinity. This graph demonstrates how Sr/Ca changes with salinity. The difference in the Sr/Ca concentrations at the freshwater and marine endmembers is not sufficient for Sr/Ca to serve as a tool for the reconstruction of salinity movements in this system.

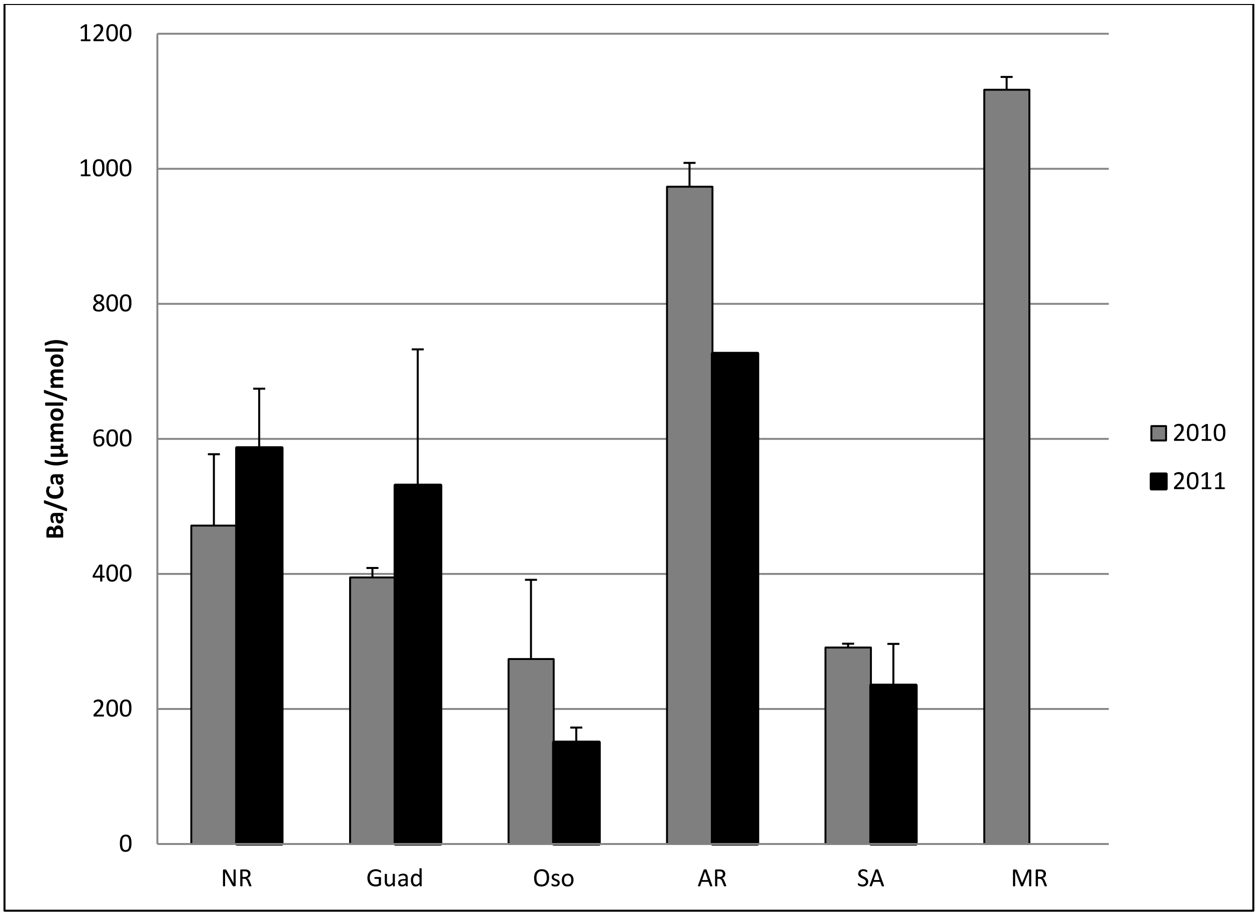


Figure 1.4. Mean $\pm$ SD freshwater Ba/Ca values (salinities less than 5) by tributary, for 2010 and 2011. This graph highlights the interannual variability in freshwater values. There are no data for the Mission River in 2011 because all sites sampled had salinities greater than 5.

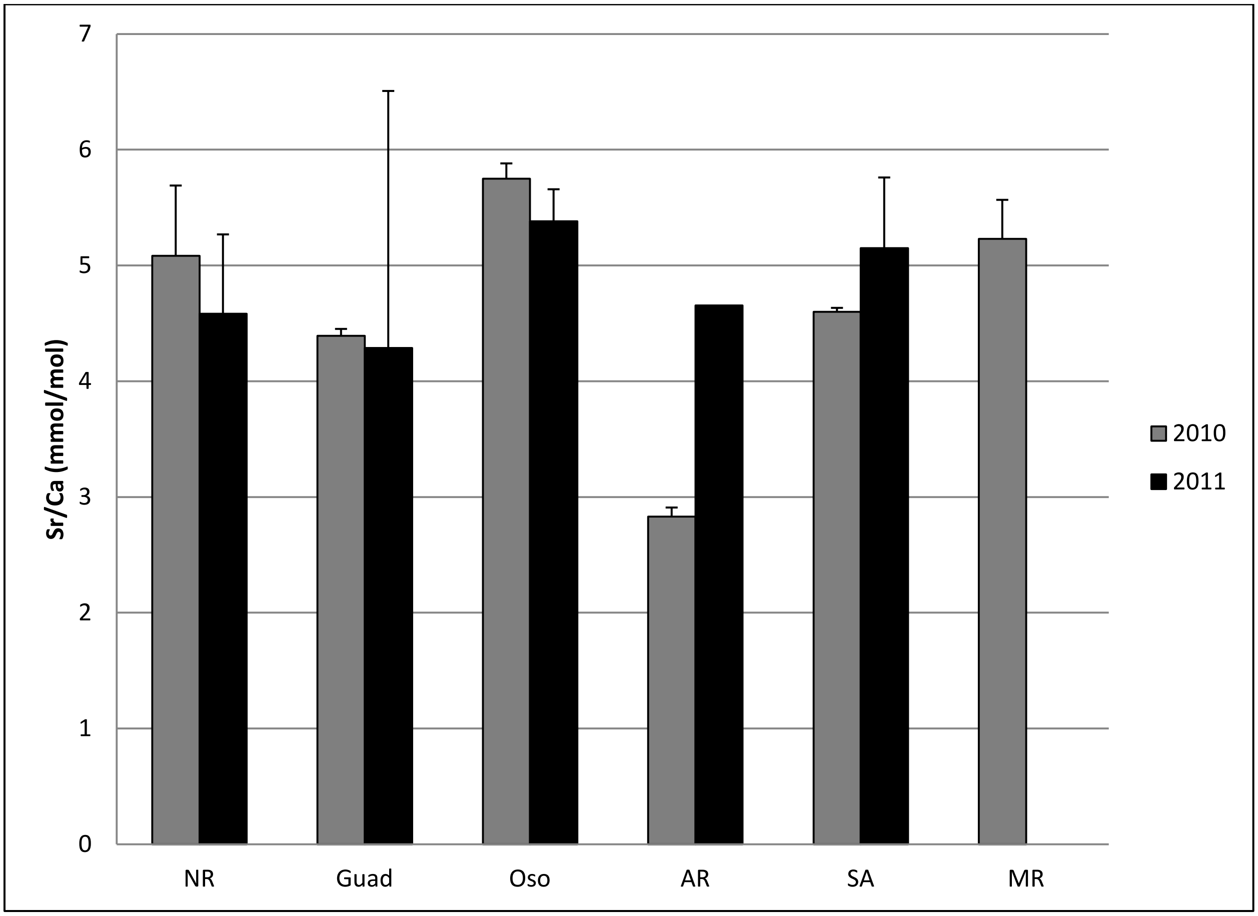


Figure 1.5. Mean $\pm$ SD freshwater Sr/Ca values (less than 5) by tributary, for 2010 and 2011. There are no data for the Mission River in 2011 because all sites sampled had salinities greater than 5.

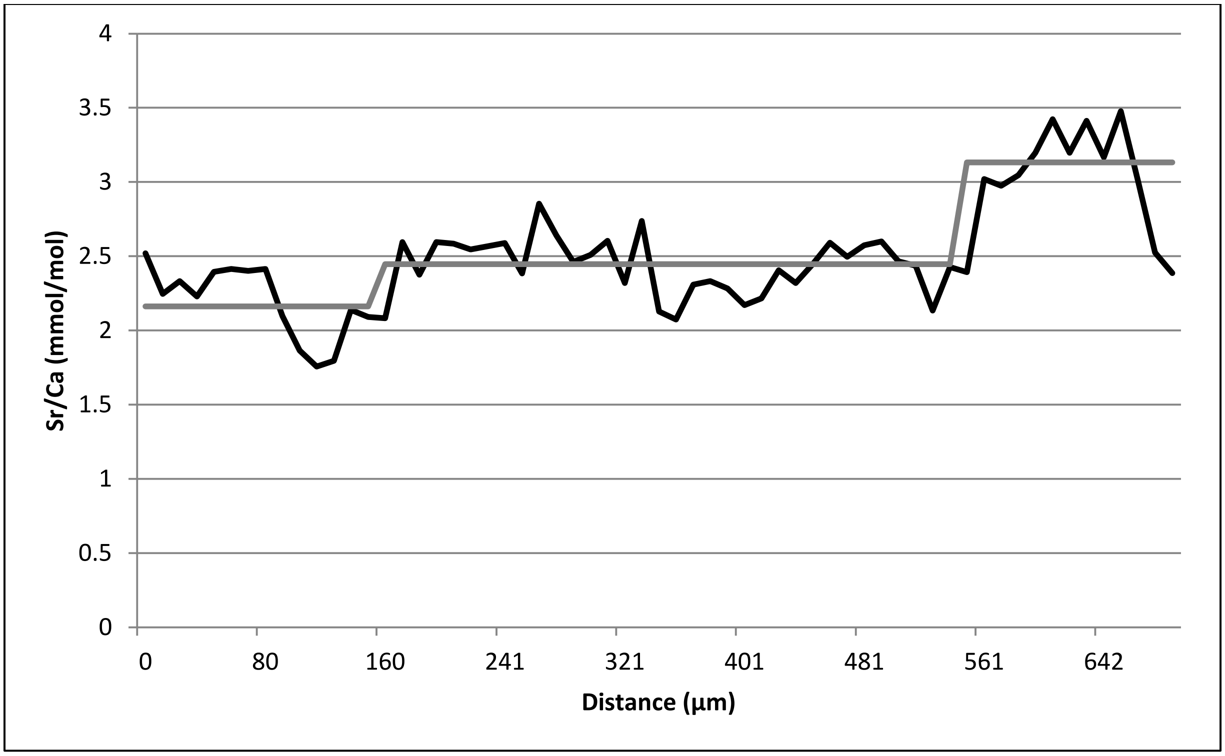


Figure 1.6. Life history transect of Sr/Ca across the otolith of individual 77. The solid black line represents the smoothed Sr/Ca data, while the grey line represents the global zoning data. The global zoning algorithm has defined significant zone differences but these differences are not able to be interpreted as salinity movements.

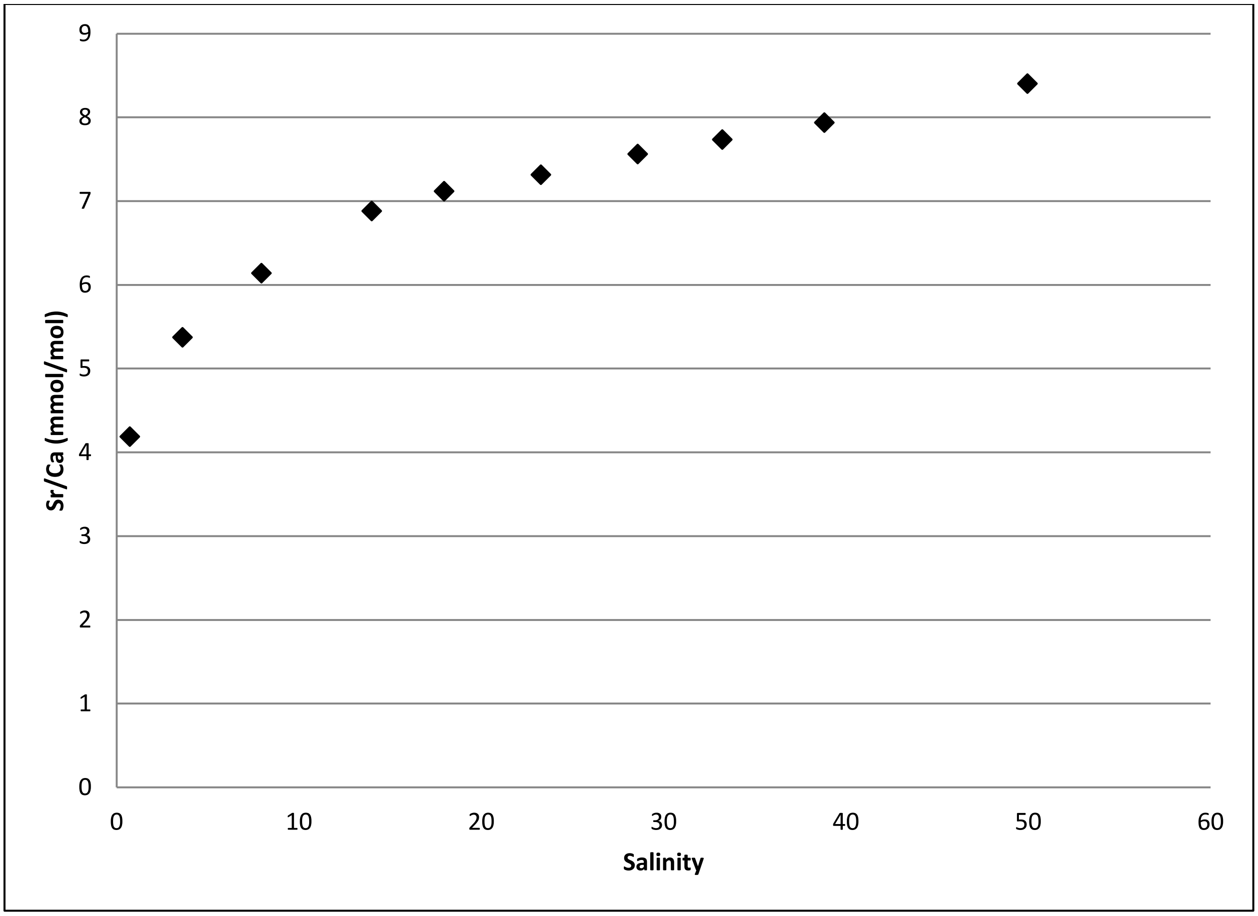


Figure 1.7. Sr/Ca variation in the Nueces River with changing salinity. Although Sr/Ca varies predictably across the salinity gradient, there is not sufficient difference between fresh and marine endmembers to make Sr/Ca a reliable tracer for the reconstruction of salinity movement patterns in South Texas.

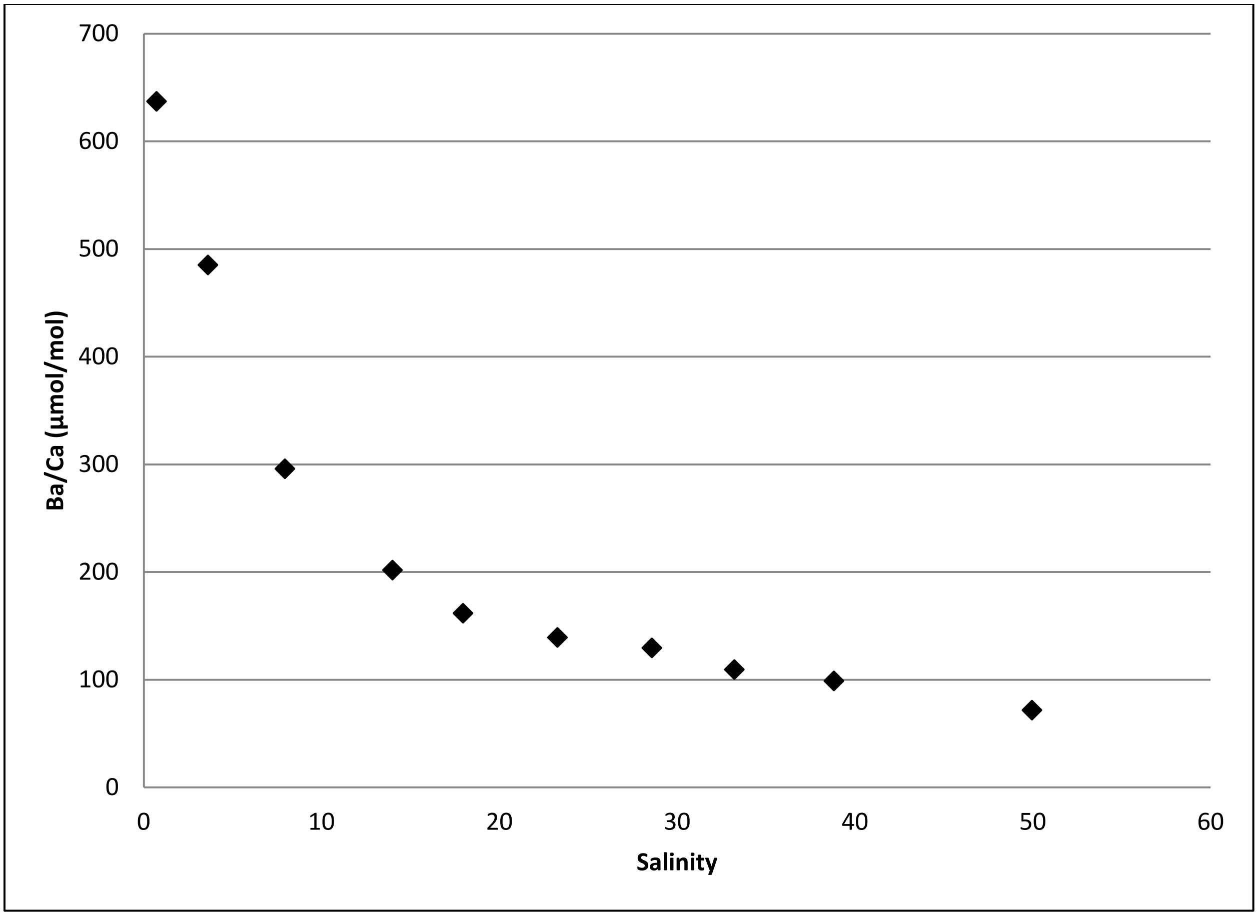


Figure 1.8. Ba/Ca variation in the Nueces River with changing salinity. The predictable variation in Ba/Ca across the salinity gradient and the difference between fresh and marine endmembers makes Ba/Ca an ideal tracer for the reconstruction of salinity movement patterns in this area.

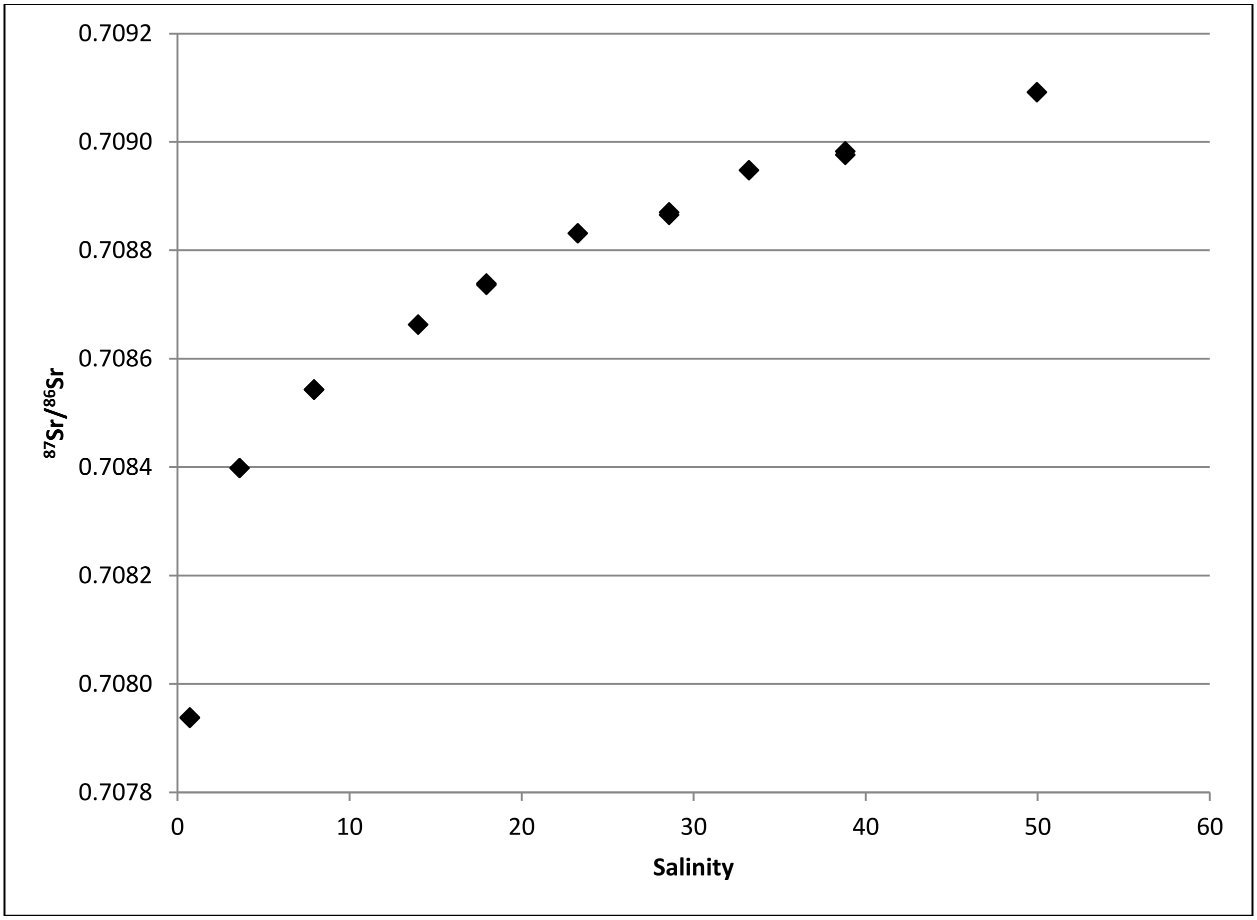


Figure 1.9. $^{87}Sr/^{86}Sr$ variation in the Nueces River with changing salinity.

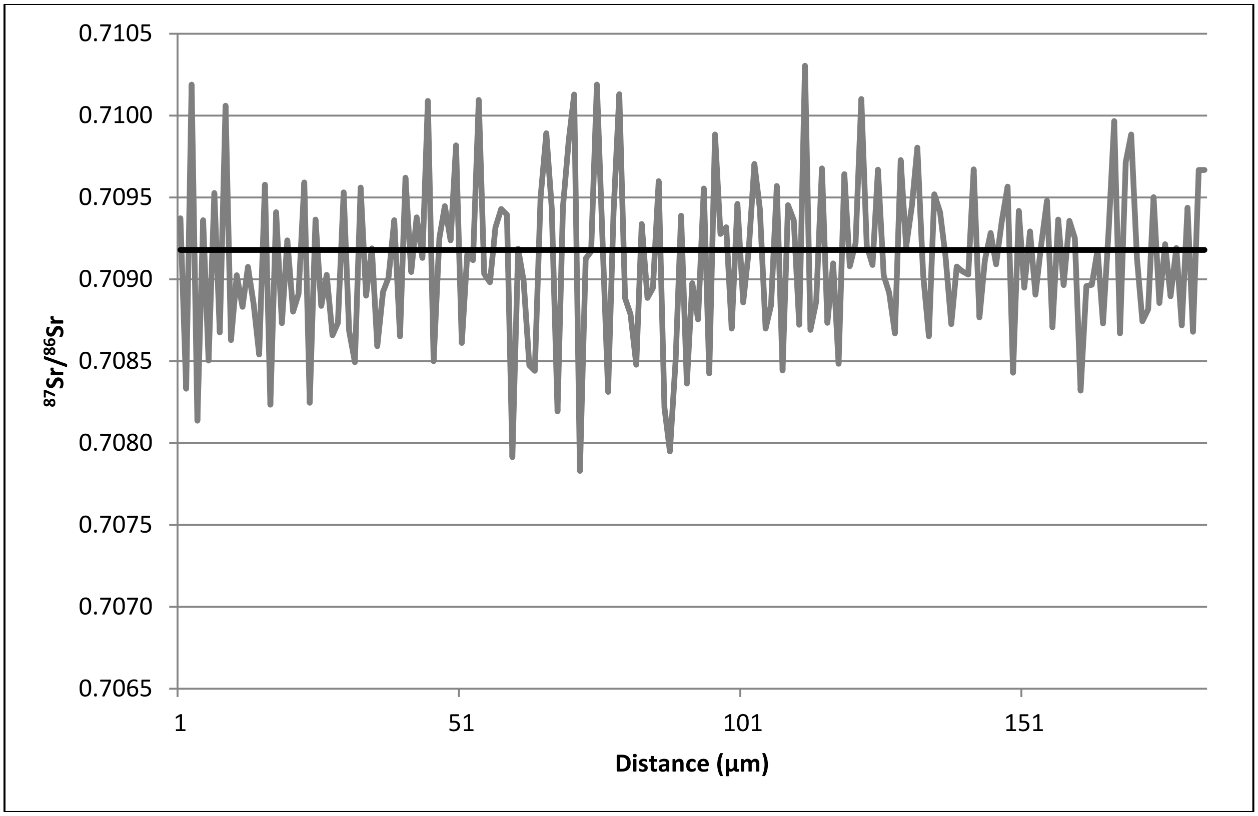
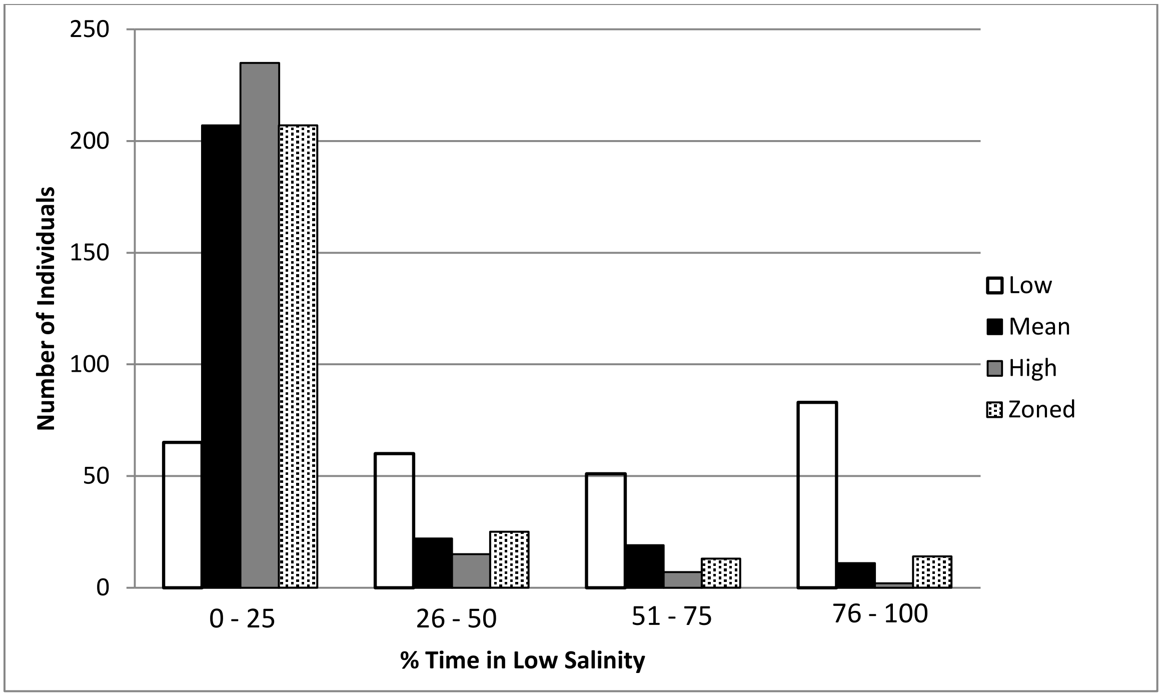
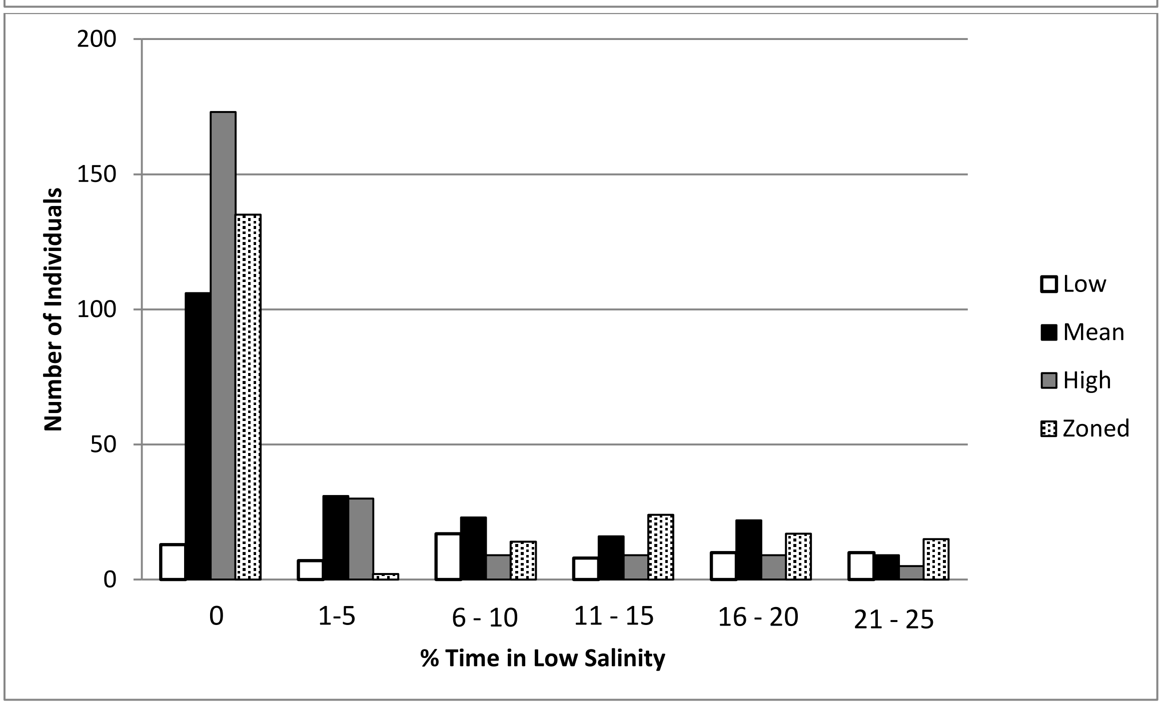


Figure 1.10. Otolith $^{87}Sr/^{86}Sr$ pattern for individual number 77. This graph is representative of the patterns seen in the majority of otoliths sampled for $^{87}Sr/^{86}Sr$. This demonstrates why $^{87}Sr/^{86}Sr$ cannot be used as a tool for salinity reconstruction in this area. The grey line represents the raw $^{87}Sr/^{86}Sr$ values. The black line represents the global marine $^{87}Sr/^{86}Sr$ value of 0.70918.

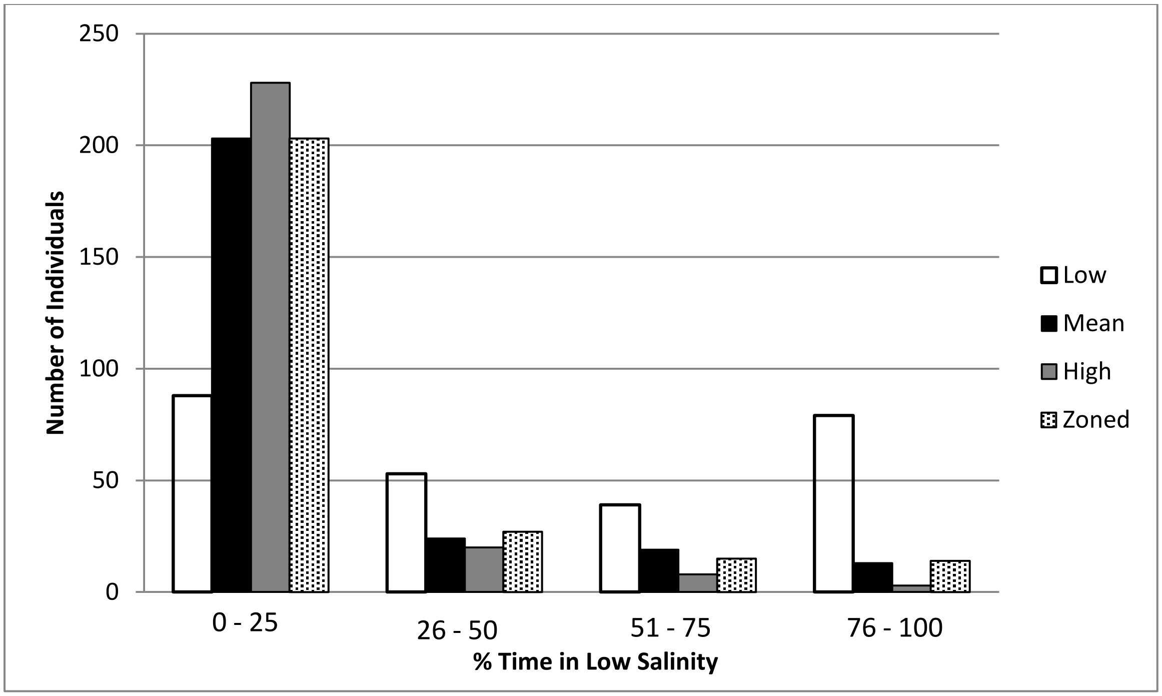


A.

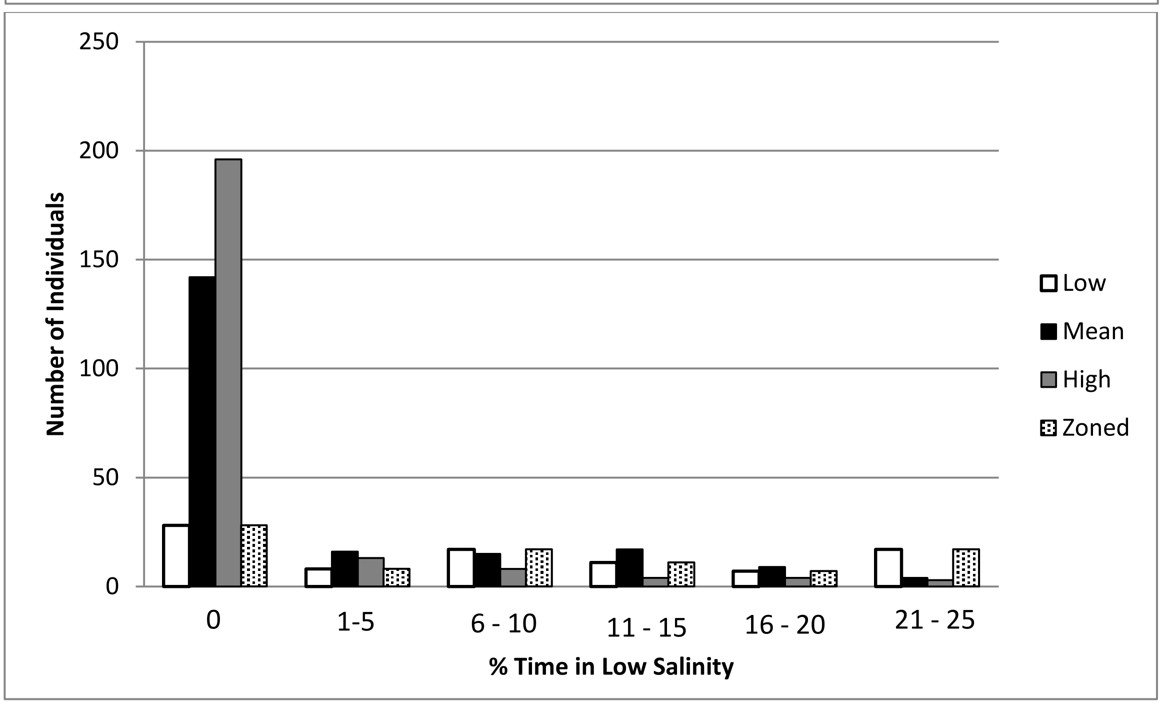


B.

Figure 1.11. A. Individuals grouped into four categories of low salinity habitat use. Data shown are for the complete life history profiles. Percent time in low salinity represents the percent of an individual's otolith transect that is categorized as low salinity, which changes depending on the threshold value used. B. The number of individuals grouped into the 0 – 25 % low salinity habitat usage category. This category is broken down further to illustrate how many individuals never enter low salinity.

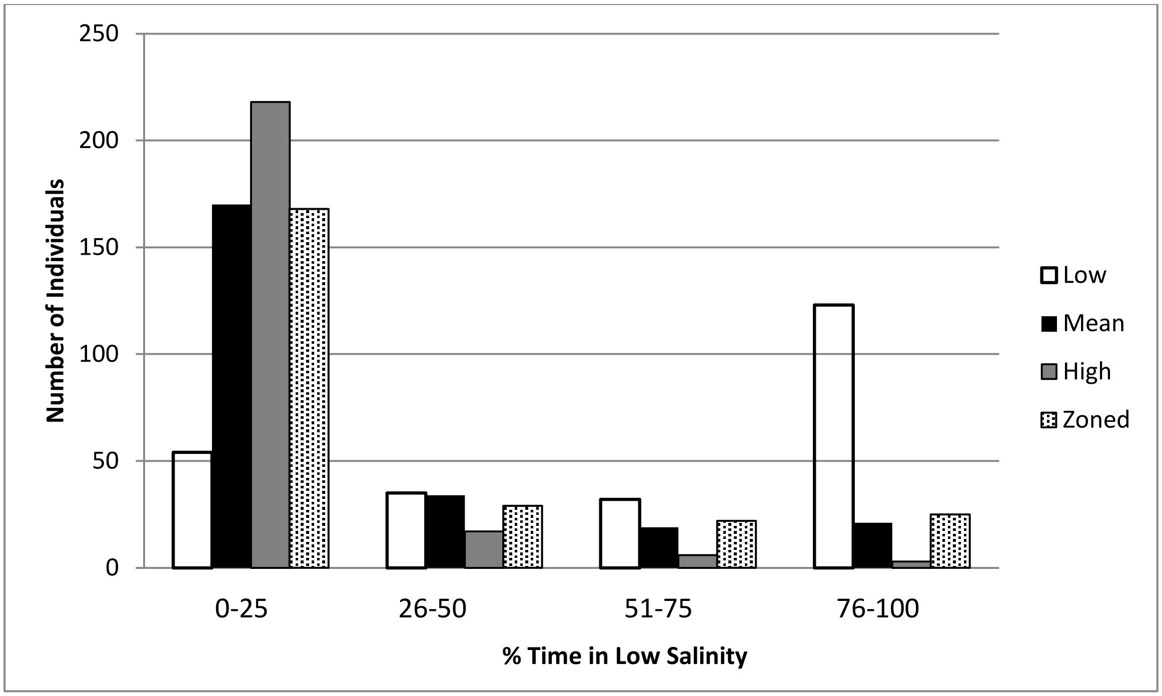


A.

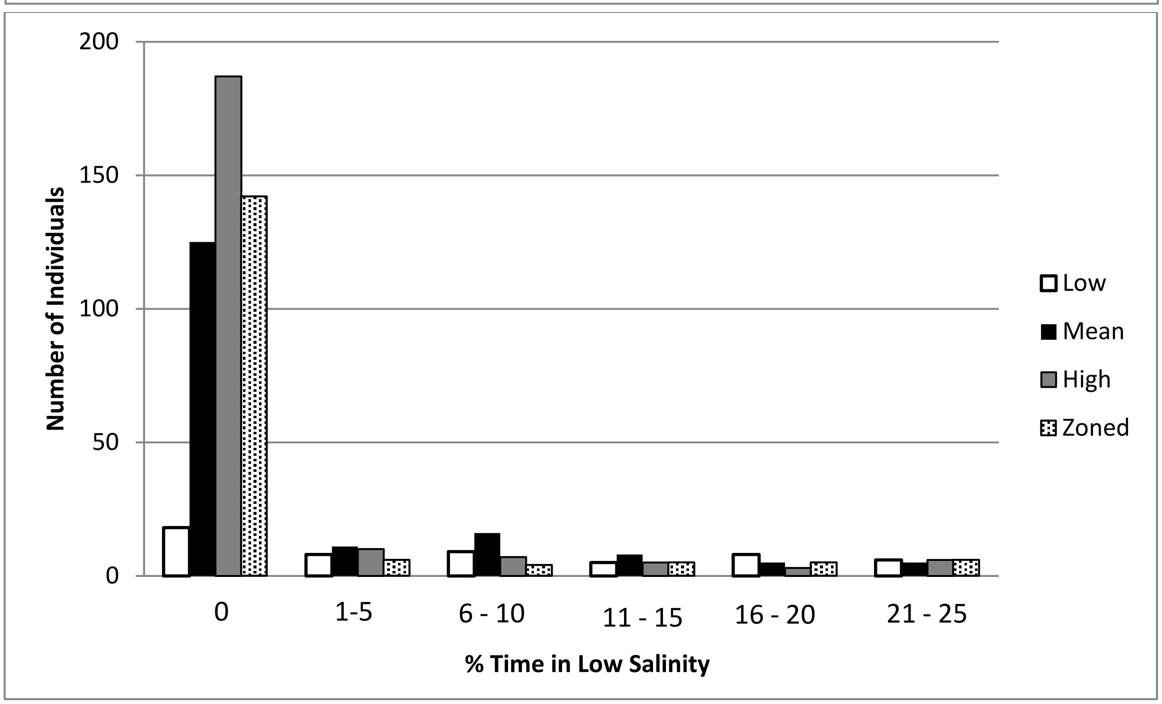


B.

Figure 1.12. A. All individuals grouped into four categories of low salinity habitat usage. Data shown here are for the first year of life only. Percent time spent in low salinity habitat represents the percent of an individual's otolith transect that can be categorized as low salinity, which changes depending on the threshold value used. B. The number of individuals grouped into the 0 – 25% usage category. This category is broken down further to illustrate how many individuals never enter low salinity.



A.



B.

Figure 1.13. A. Individuals (age ≥ 1 yr) that were grouped into four categories of low salinity habitat use. Percent time spent in low salinity habitat represents the percent of an individual's otolith transect was categorized as low salinity, which changes with the threshold value used. The data here are the percent of the transect after the year 1 annulus. B. The number of individuals grouped into the 0 – 25% usage category.

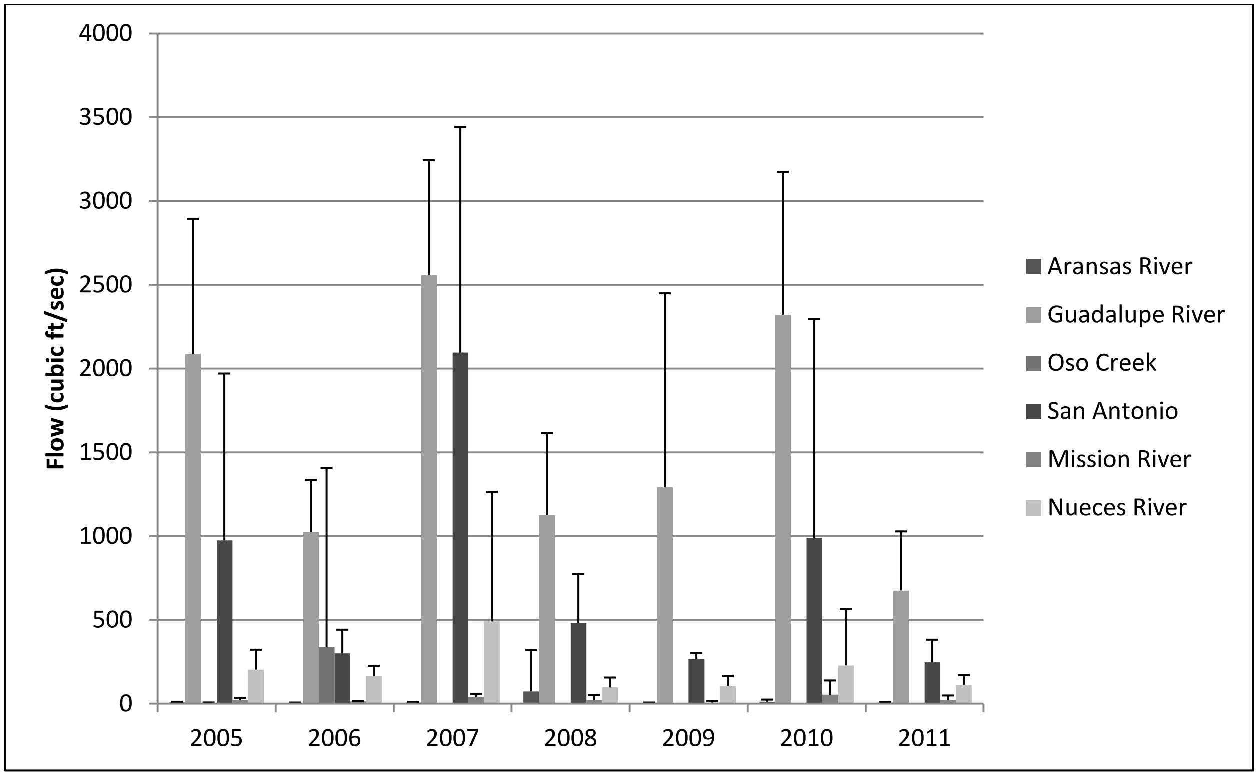


Figure 1.14. Mean annual + SD stream flow (measured instantaneously in cubic feet/second) for each of the six tributaries sampled in this study for 2005 – 2011 (Data from the Nueces River Authority).

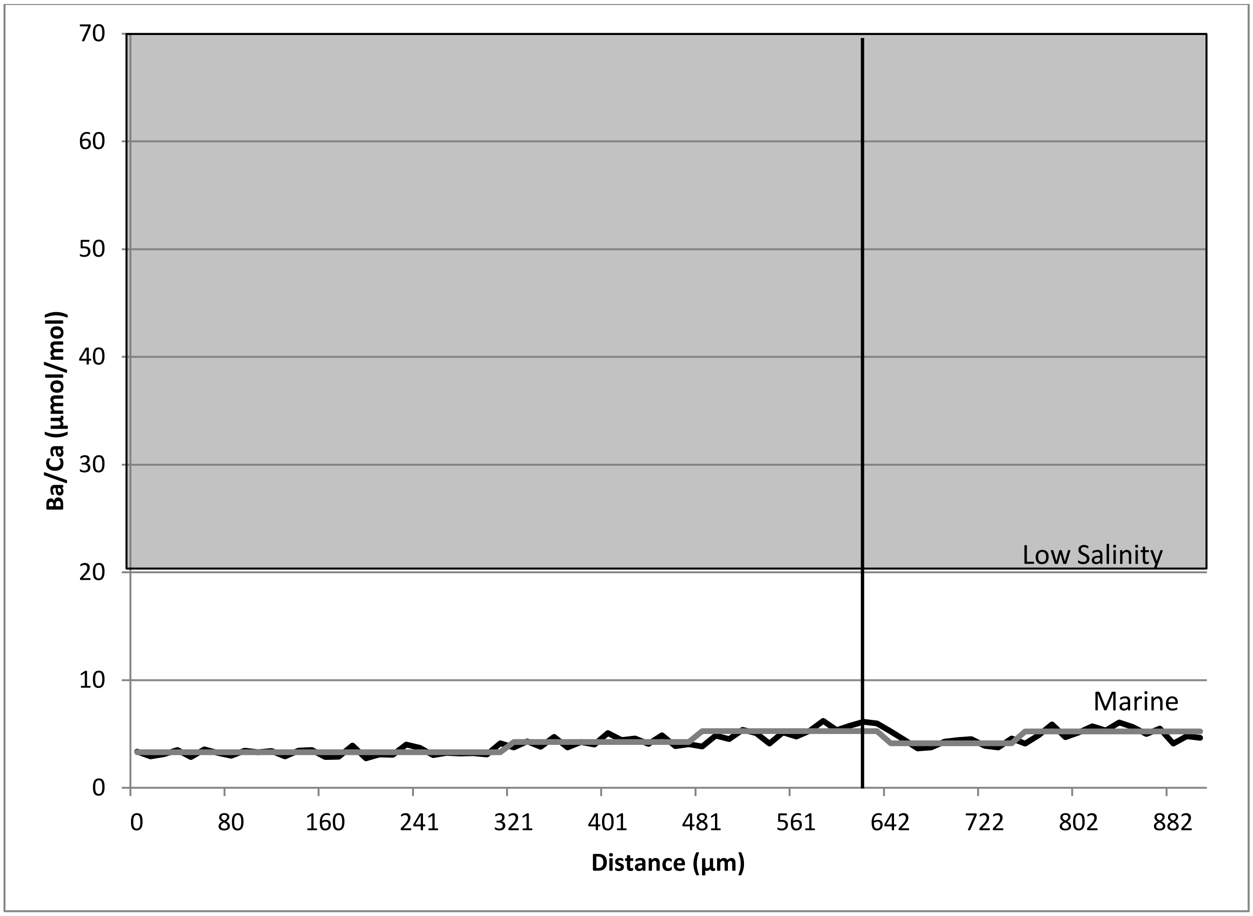


Figure 1.15. Individual number 7 exhibits very low Ba/Ca concentrations throughout the otolith. This is consistent with an individual that has spent its entire life in the marine environment. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data. The black vertical line represents the position of the first annulus.

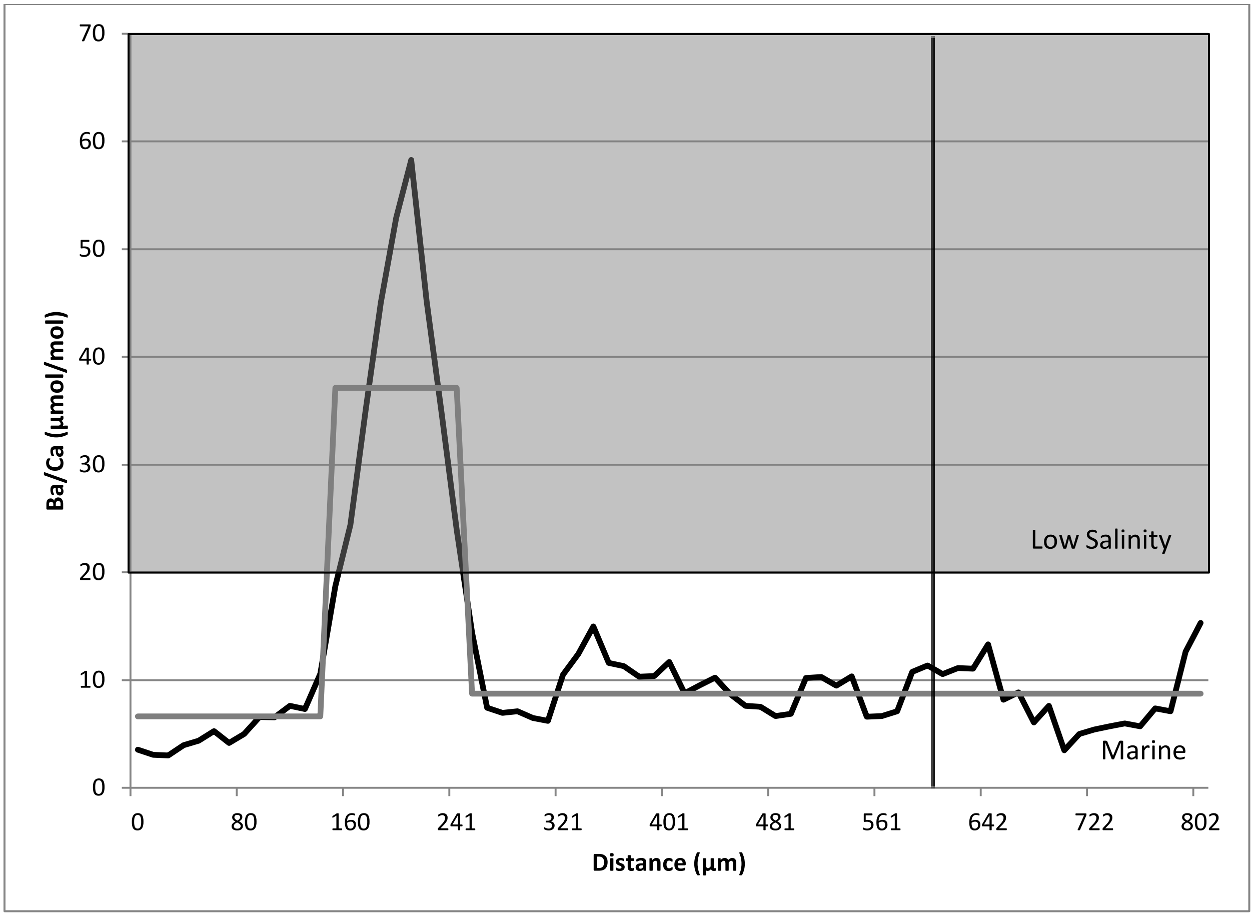


Figure 1.16. Individual number 15 exhibits a Ba/Ca pattern that is consistent with the early life stages spent in the marine environment, followed by a movement into low salinity habitat . The fish then returns to the marine environment for the remainder of its life. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data. The black vertical line represents the position of the first annulus.

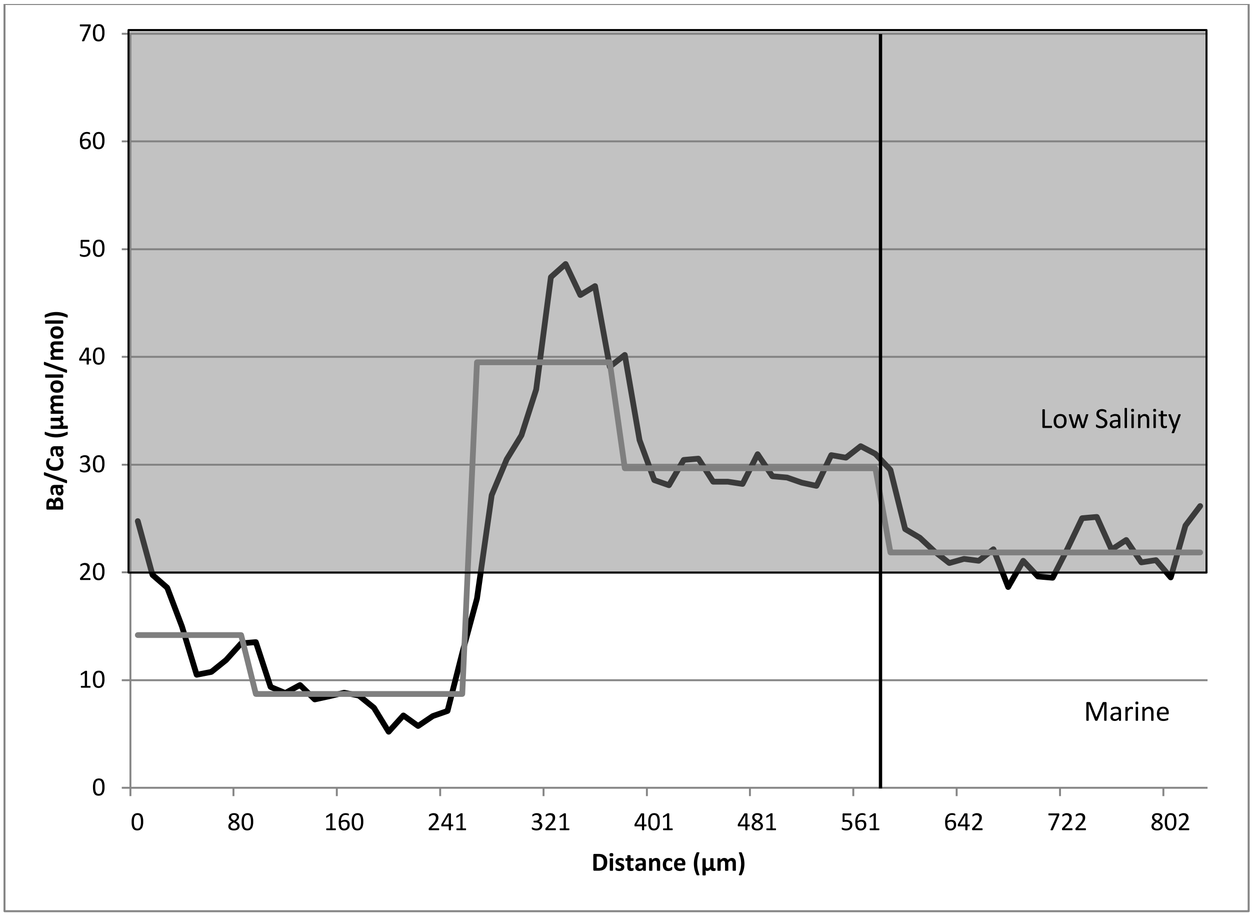


Figure 1.17. Individual number 37 exhibits a Ba/Ca pattern that is consistent with the early life stages spent in the marine environment, followed by a movement into low salinity habitat. The fish then remains in low salinity habitat for the duration of its life. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data. The black vertical line represents the position of the first annulus.

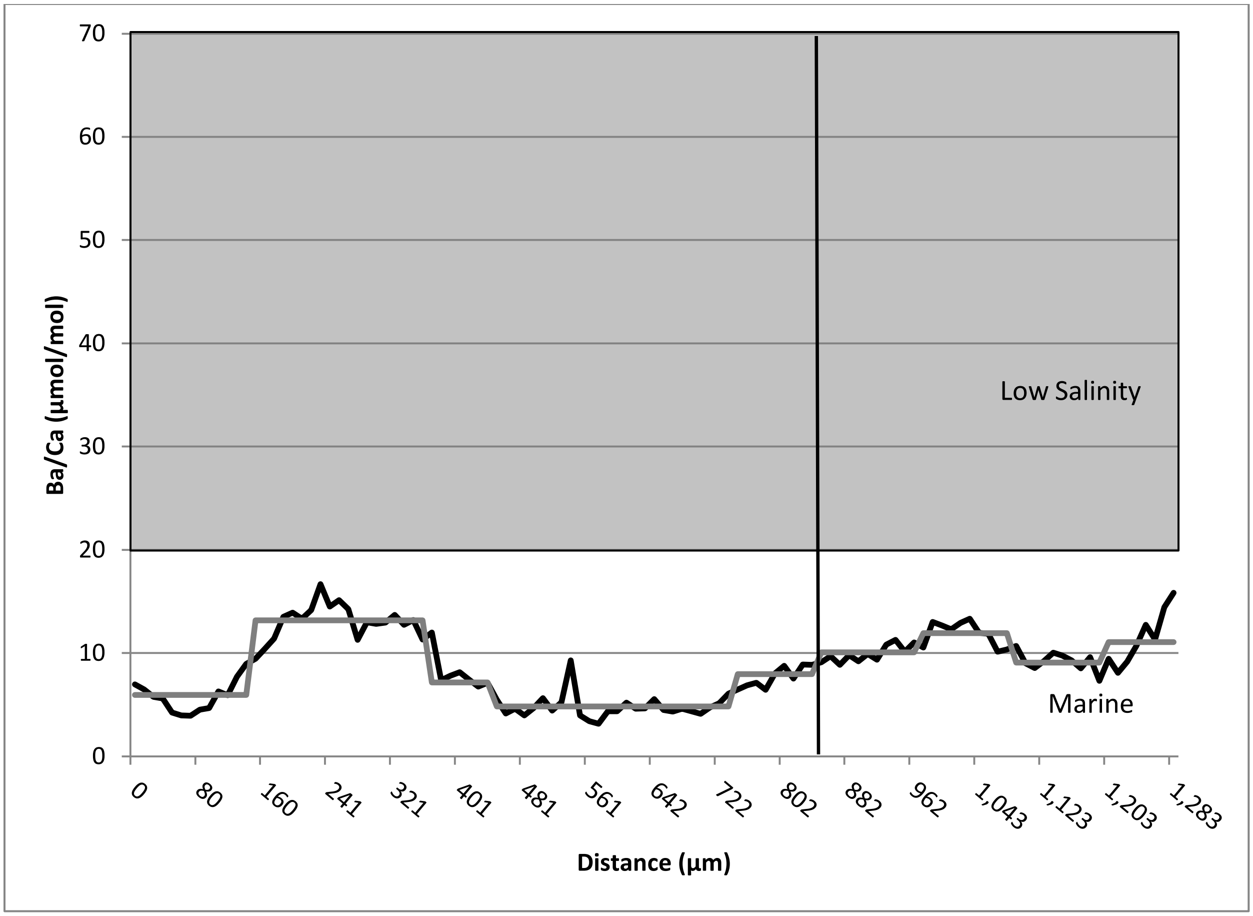


Figure 1.18. Individual number 42 spends its early life stages in the marine environment before making an excursion into the estuarine environment. This fish then returns to the marine environment for a period of time before returning to an estuarine environment. Although this fish never enters low salinity habitat, it is clear that there is variability in habitat use patterns throughout the individual's life. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data. The black vertical line represents the position of the first annulus.

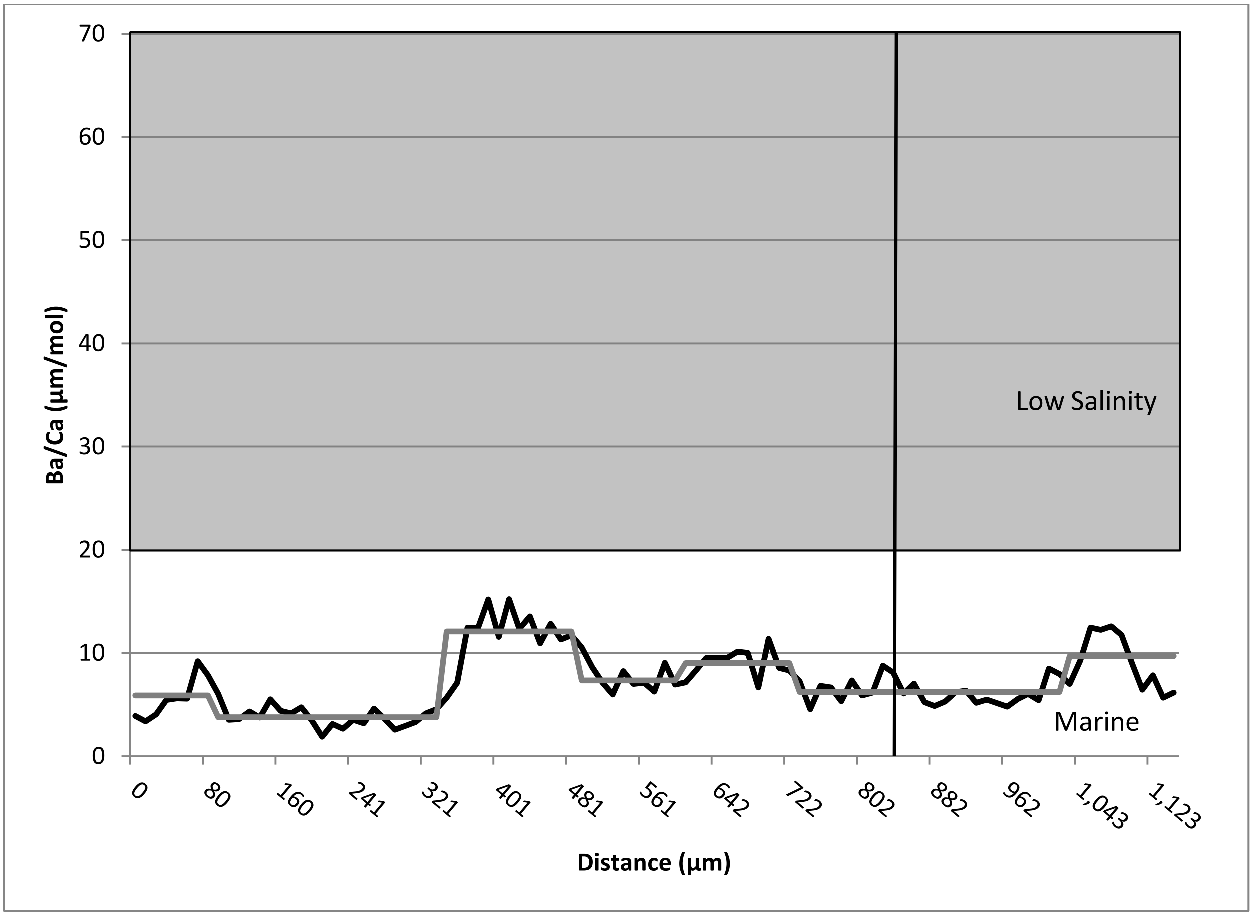


Figure 1.19. Individual number 16 spends its early life stages in the marine environment before making an excursion into the estuarine environment. This fish then returns to the marine environment for the remainder of its life. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data. The black vertical line represents the position of the first annulus.

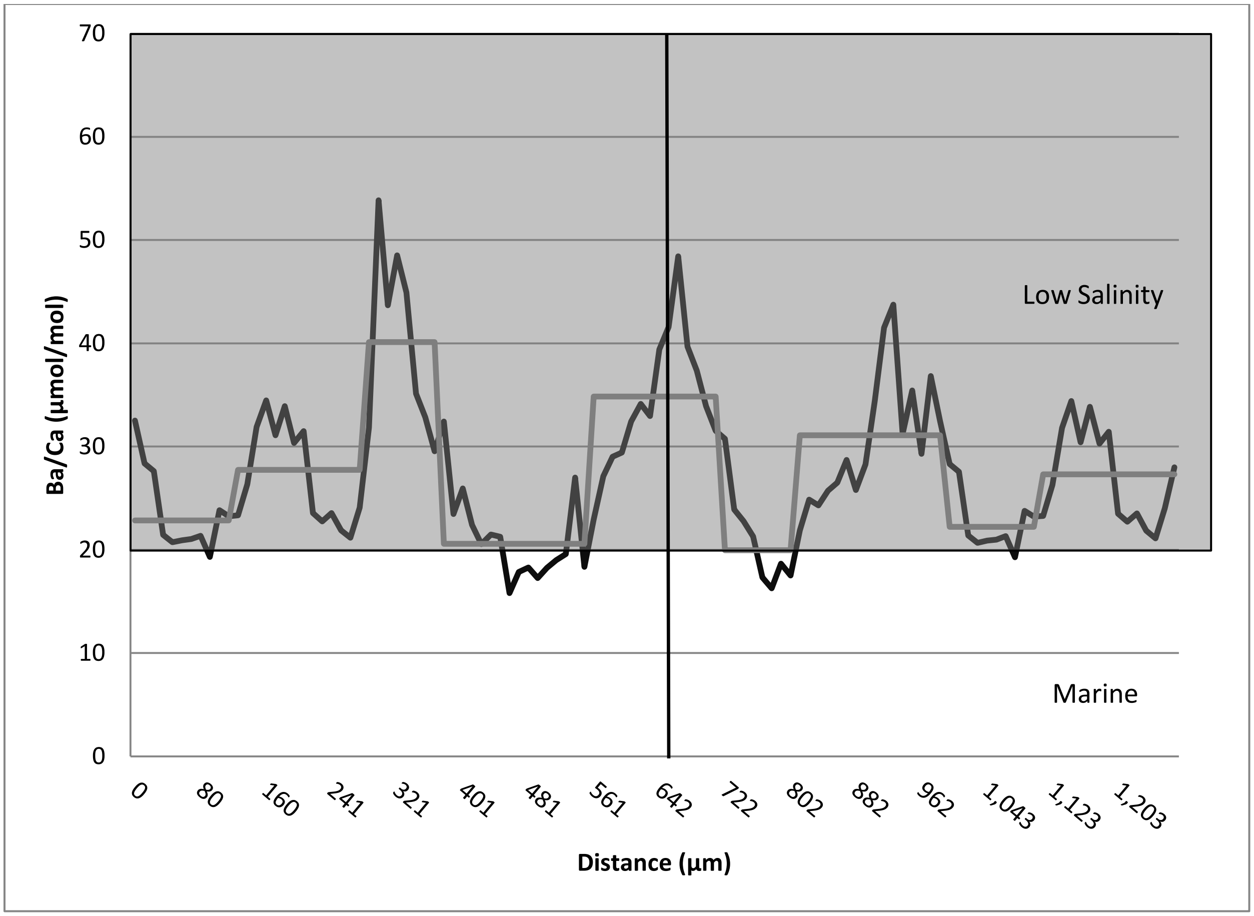


Figure 1.20. Individual 83 exhibits high Ba/Ca concentrations early in life. Usually, this is consistent with residency in low salinity habitat. It is likely that this fish was hatched in high salinity water and then moved into fresh or low salinity water very quickly after hatching. This fish remained in a low salinity environment for its entire life. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data. The cyclic pattern exhibited in this otolith could indicate that this individual was making repeated movements into the estuarine or marine environment but these movements were too rapid for the otolith to fully equilibrate to the new salinity environment. The black vertical line represents the position of the first annulus.

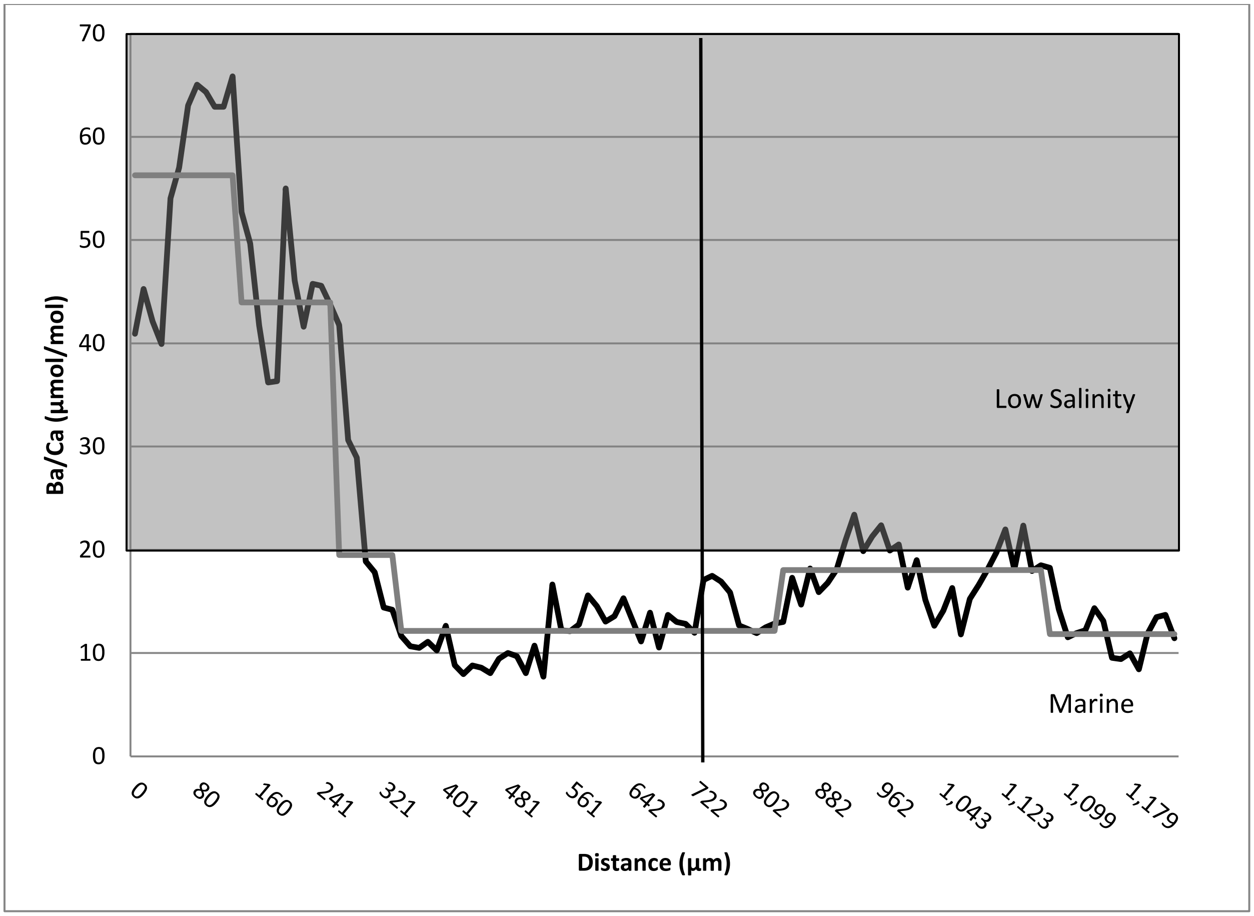


Figure 1.21. Individual 54 exhibits high Ba/Ca concentrations early in life, consistent with residency in low salinity habitat. It is likely that this fish was hatched in high salinity water and then moved into fresh or low salinity water very quickly after hatching. This fish then moved to the estuarine environment for most of the remainder of its life. The solid black line represents the smoothed Ba/Ca data, while the grey line represents the global zoning data.

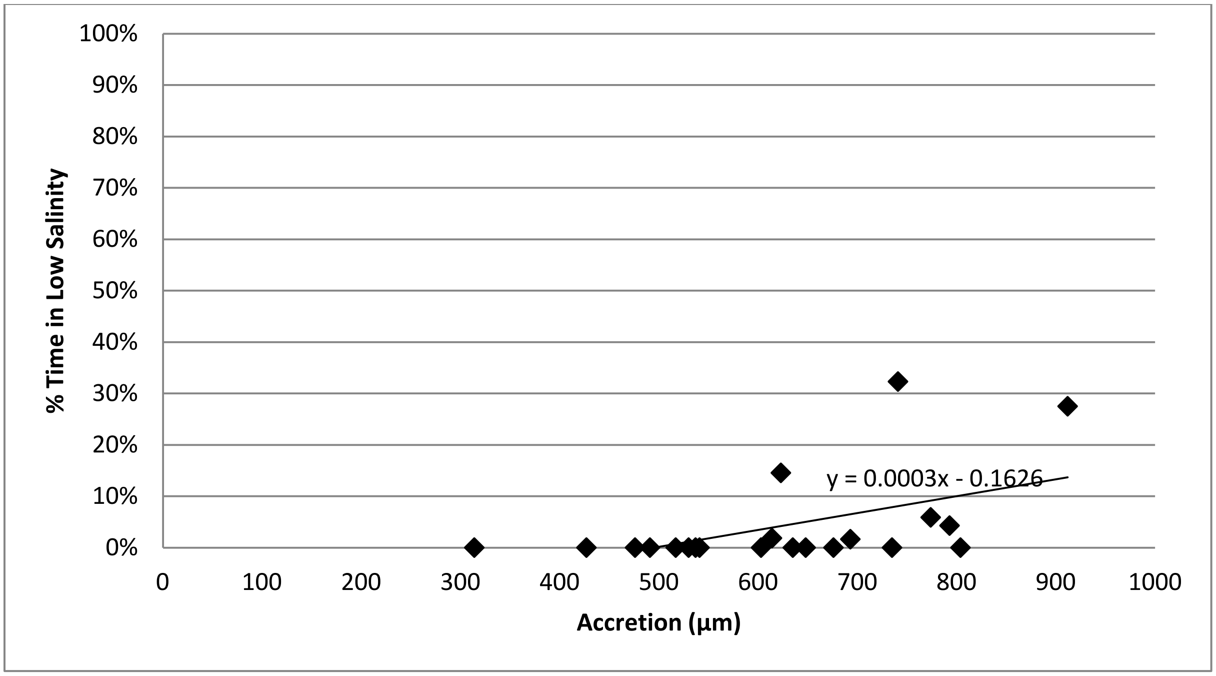


Figure 1.22. The percent of an individual's otolith transect (data from year 1 portion of the transect only) classified as low salinity plotted against otolith accretion for fish age 0. For this analysis, the mean threshold of 20 µmol/mol Ba/Ca was used. The slope of the regression line is significantly different from zero (p=0.02).

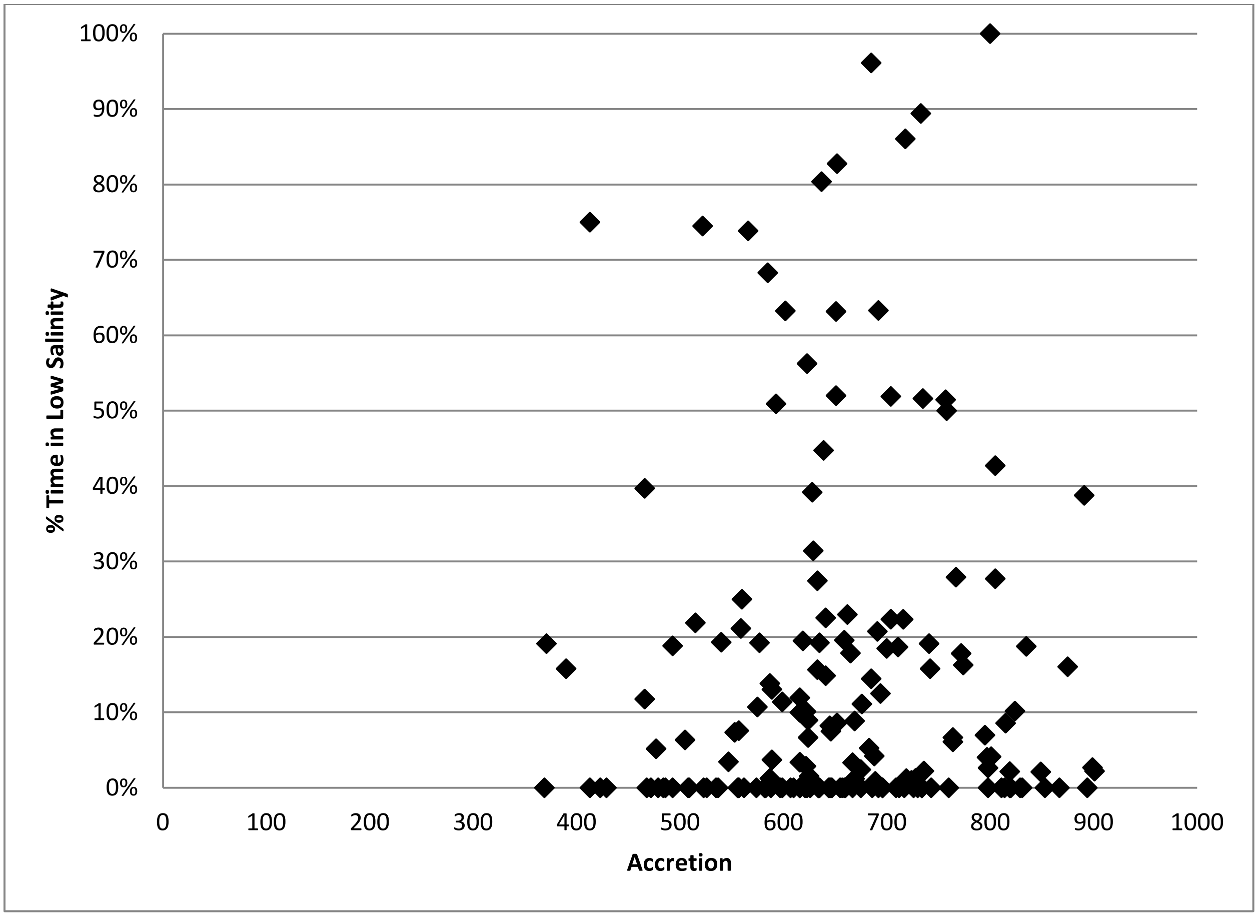


Figure 1.23. The percent of an individual's otolith transect (data from year 1 portion of the transect only) classified as low salinity plotted against otolith accretion during year 1 for fish age 1. For this analysis, the mean threshold of 20 μmol/mol Ba/Ca was used. No significant relationship was found.

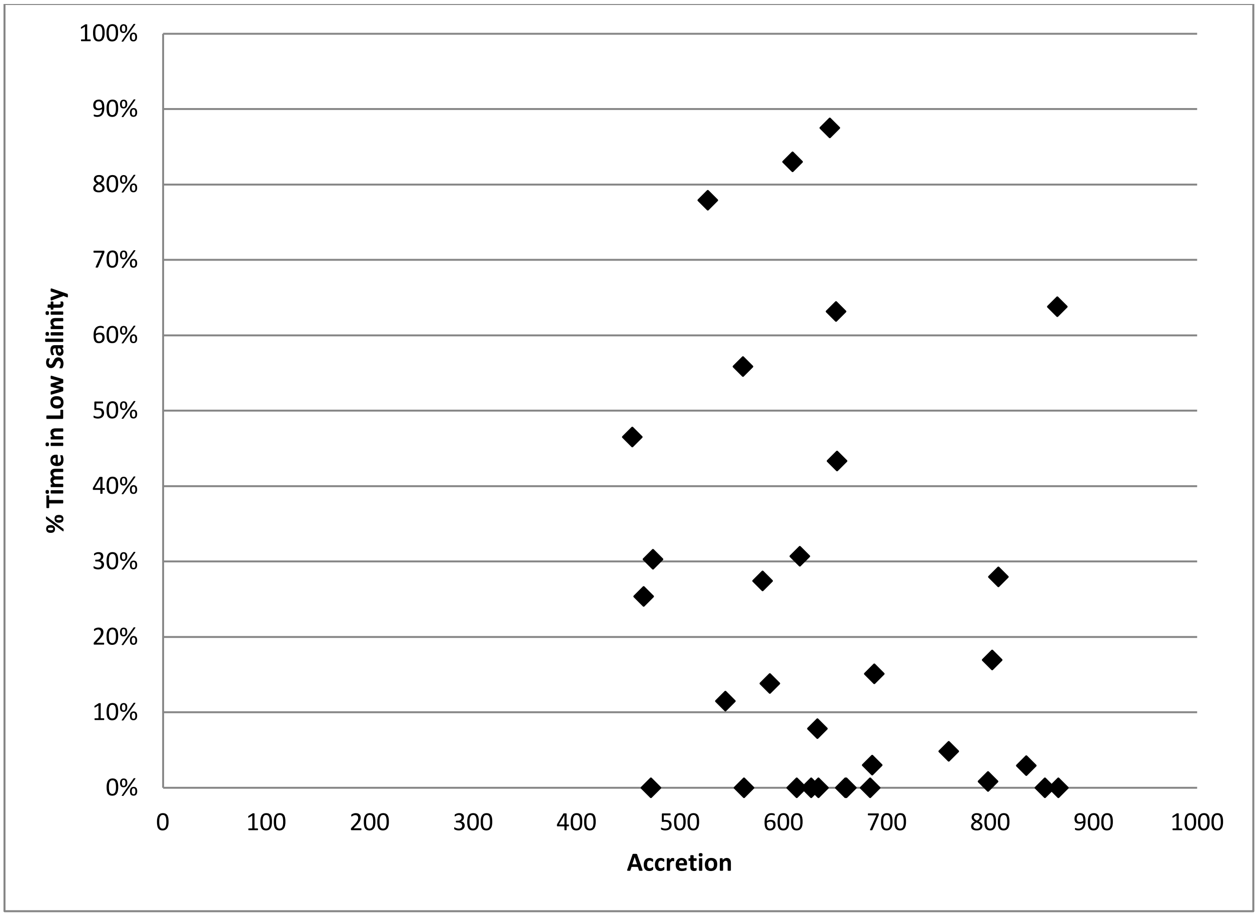


Figure 1.24. The percent of an individual's otolith transect (data from year 1 portion of the transect only) classified as low salinity plotted against otolith accretion during year 1 for fish age 2. For this analysis, the mean threshold of 20 µmol/mol Ba/Ca was used. No significant relationship was found.

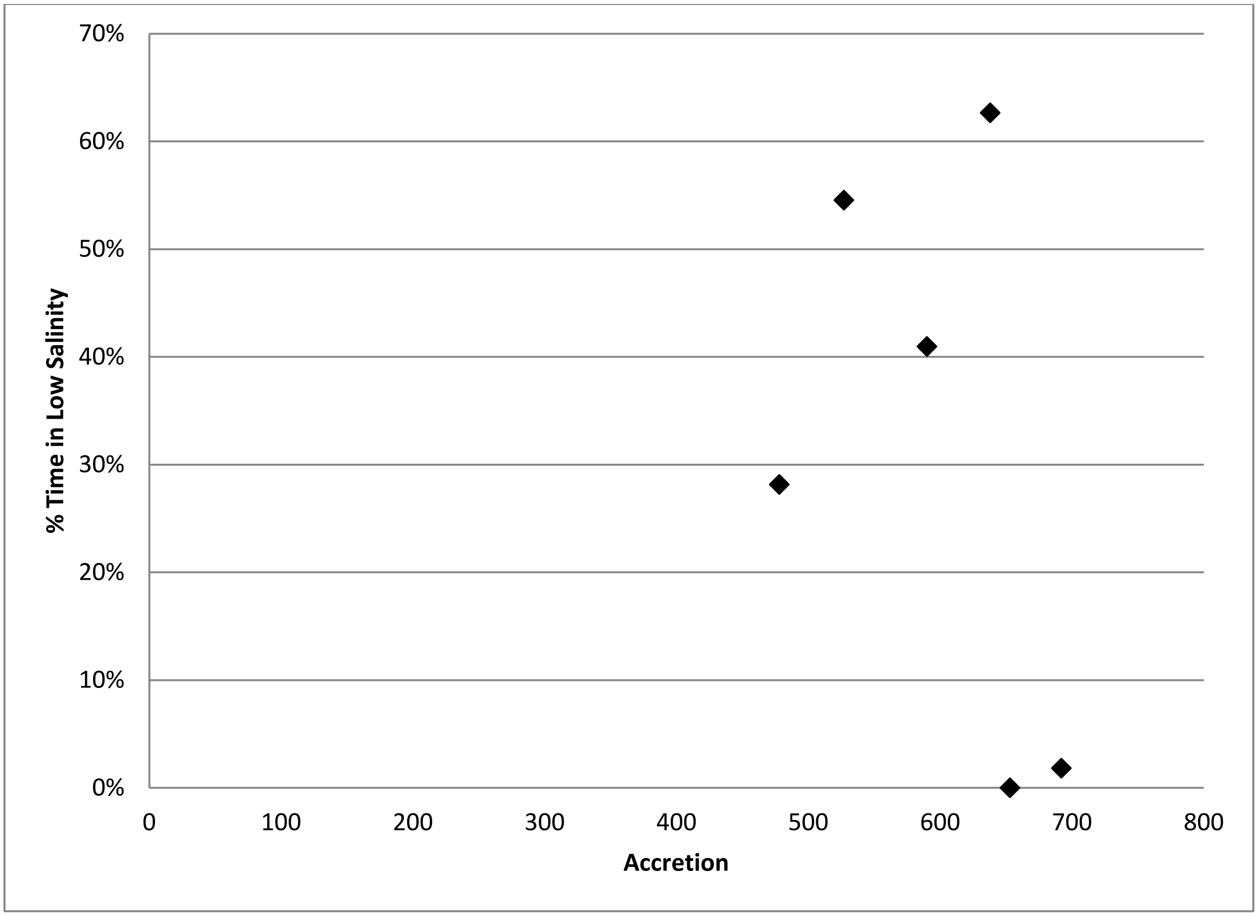


Figure 1.25. The percent of an individual's otolith transect (data from year 1 portion of the transect only) classified as low salinity plotted against otolith accretion during year 1 for fish age 3. For this analysis, the mean threshold of 20 µmol/mol Ba/Ca was used. No significant relationship was found.

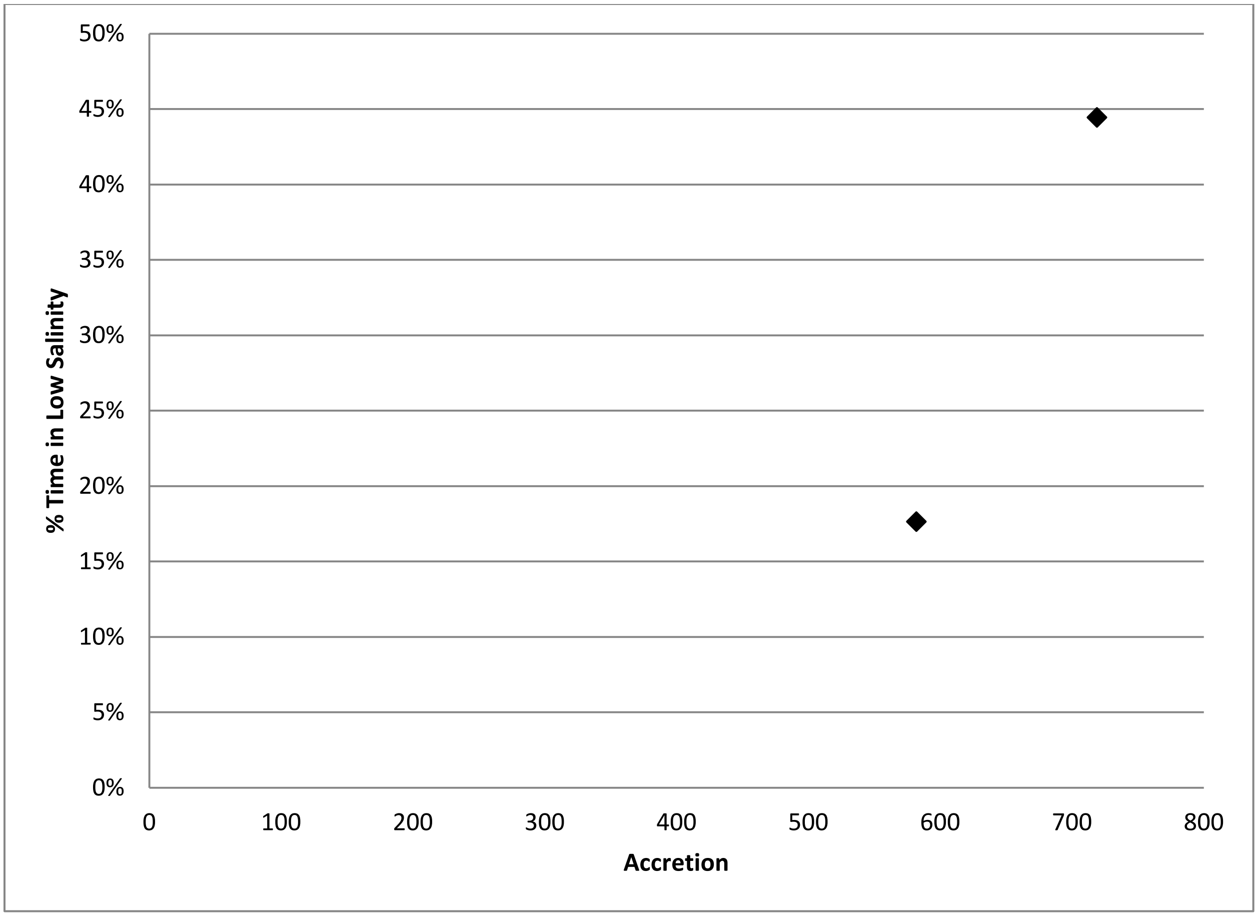


Figure 1.26. The percent of an individual's otolith transect (data from year 1 portion of the transect only) classified as low salinity plotted against otolith accretion during year 1 for fish age 4.

CHAPTER 2: OTOLITH EQUILIBRATION RATES IN SOUTHERN FLOUNDER UNDER CHANGING SALINITIES

Introduction

Otolith microchemistry is a popular tool for the reconstruction of environmental conditions, particularly salinity, that an individual fish experiences during its lifetime. Otoliths are metabolically inert and grow continuously throughout a fish's life making them ideal tools for reconstructing the life history of a fish. However, there are many factors that influence the uptake and incorporation of elements into otoliths that must be considered when interpreting chemical patterns seen in otoliths. First to consider is the complicated pathway in which elements are incorporated into the otolith. Ions pass across either gill or intestinal epithelia to the blood plasma, and then across a membrane into the endolymph surrounding the otoliths, and lastly, ions are incorporated into the otolith in the crystal matrix itself or adhered to the organic scaffolding that directs crystal growth (Campana 1999). Elemental discrimination can occur at each of these three barriers (epithelia, endolymph membrane, crystal surface), which potentially results in a significantly smaller amount of an element incorporating into the otolith as compared to the concentration of that particular element in the water (Campana 1999). The proportion of the element (Me) that is found in the otolith in comparison to the concentration of the element in the water is called the partition coefficient (D_{Me}) and is calculated using the formula (Morse and Bender 1990):

$$D_{Me} = \frac{(Me{:}Ca)_{otolith}}{(Me{:}Ca)_{water}}$$

Temperature (Bath et al. 2000), salinity (Miller 2011), interaction or facilitation with other elements (De Vries et al. 2005), and physiological processes (Kalish 1989; Walther et al. 2010) have all been demonstrated to have an impact on elemental uptake and incorporation into otoliths. However, some elements in otoliths, such as strontium (Sr) and barium (Ba), have been shown to be overwhelmingly dominated by elemental concentrations in the ambient water (Bath et al. 2000; Walther and Thorrold 2006). Because of the continuous growth of otoliths over the lifetime of the fish, exposure to a new ambient concentration of elements like Sr and Ba leads to eventual equilibration in the otolith to the new elemental concentration. Yet, because of the complicated ionic pathways and potential for discrimination described above, there can be a time lag between when a fish is exposed to a particular elemental concentration in the water and when that element appears at equilibrium in the otolith.

Previous research suggests that this time lag can be extensive, which is significant when interpreting the movement patterns of individual fish on finer time scales. Elsdon and Gillanders (2005) demonstrated that it could take at least 20 days for otolith Sr concentrations to reach full equilibrium after black bream (*Acanthopagrus butcheri)* were exposed to significant shifts in Sr. Yokouchi et al. (2011) found that in Japanese eels (*Anguilla japonica*) changes to otolith Sr concentrations were not detected until 10 days following a change in Sr concentrations. A model predicted that this species would not reach otolith equilibration until 30 – 60 days following a shift in Sr concentration. Similarly, Miller (2011) found that following a significant shift in Ba concentrations, changes in otolith Ba concentrations of Chinook salmon (*Oncorhynchus tshawytscha*) could be detected within 2 – 3 days but Ba otolith concentrations did not equilibrate for 12 – 14 days. These prior results suggest that fish movements across elemental gradients at high frequencies (less than 15-30 days) may be less evident in elemental life history profiles from otoliths than longer-term shifts in habitat residence. Although previous research has been conducted examining the response time of otoliths in response to changing Sr and Ba, elemental incorporation rates and partition coefficients are species-specific, and research investigating these processes in flatfish is lacking.

As demonstrated in Chapter 1, southern flounder on the Gulf Coast of Texas exhibit variable habitat use throughout their juvenile period. A more complete understanding of the dynamics of Ba uptake in southern flounder is needed to better understand and interpret the patterns seen in the otoliths from wild fish. A laboratory experiment was conducted to investigate 1.) the partition coefficient of Ba (D_{Ba}) and 2.) otolith equilibration rates of Ba under changing salinities.

Materials and Methods

Experimental Design

Adult southern flounder were collected from the Lydia Ann Channel near Port Aransas, Texas in the fall of 2010. Temperature and photoperiod were manipulated, per Arnold et al. (1978), to induce spawning of captured adult southern flounder. Resulting eggs were incubated and reared through metamorphosis following procedures detailed in Faulk and Holt (2009) and Daniels (2000). Juveniles were reared until they were at least 9.0 cm total length (TL). All juveniles were reared in seawater that varied in salinity from 35 – 40. Prior to the experiment, juveniles were separated and measured and only individuals between 9.0 – 14.5 cm TL were used for the experiment. A reciprocal transplant experiment was designed to investigate the otolith equilibration rates and D_{Ba} (Table 2.1).

To visually mark the beginning of the experiment in the otolith, all juvenile southern flounder were immersed in a solution of Alizarin Red S (ARS) (400 mg/L), using procedures detailed in Liu et al. (2009). Fish were starved for 24 h prior to immersion in the ARS bath. After 24 h in the ARS solution, juveniles were removed and rinsed with filtered seawater, and placed in water with a salinity of 35 for 24 h. Since the ARS treatment is very stressful for the fish and the 24 h in water with a salinity of 35 was designed to let the fish rest before acclimation. After the rest period, juveniles were then acclimated to their respective salinities over 24 h.

Juveniles were held in independently-recirculating 20-L tanks and were exposed to one of three salinities (0, 15, 35) for 30 days. Eight fish were randomly assigned to each treatment tank, with 6 replicate tanks for each phase one salinity treatment. Salinity and water temperature was recorded daily. Fish were fed daily 5% of their body weight (mean wet weight of a random sample of 10 fish at the beginning of the study) (Daniels 2000), with excess food being siphoned from the tanks daily. After 30 days, juveniles were again marked with ARS using the above protocol to visually mark the end of phase one of the experiment in the otolith. After the 24 h rest period, juveniles were acclimated to their prescribed phase two salinity. The original experimental design called for the fish to be raised for another 30 days in the second salinity treatment and mark the otoliths using ARS to mark the end of the experiment, followed by a 10 day grow-out period. However, no individuals survived to the end of the second experimental phase.

When there was a fatality in a tank, the fish was removed and measured and otoliths were extracted from fish, cleaned, sectioned and mounted on petrographic slides according to procedures detailed by Kraus and Secor (2004). Five left otoliths were randomly subsampled to be analyzed from each tank. However, due to cannibalism, otolith loss, breakage, and other otolith processing issues, there were two tanks (4 and 16), where there were less than 5 fish available per tank. Prior to analysis, otoliths were cleaned using sonication under a class-100 laminar flow hood. Slides containing the otoliths were first dipped in ultrapure (18.0 MΩ.cm) water and then scrubbed for one minute using a soft-bristled toothbrush. The slide was then transferred to a plastic, acid washed beaker, covered with ultrapure water and placed in a sonicator. After two minutes, the slide was removed and triple rinsed with ultrapure water. Slides were then air dried and sealed in plastic petri dishes for transport.

Water samples were taken twice per week throughout the experiment. Water samples were collected using acid-washed polytetrafluorethylene (PTFE) syringes and filtered with PTFE 0.45 μm and 0.20 μm filters. Water samples were stored in 60 mL acid-washed LDPE bottles. Water samples were fixed in 2% trace metal grade nitric acid after collection and refrigerated until analysis. One water sample per week was chosen to be analyzed.

Sample Analyses

Water and otolith samples were analyzed at the Jackson School of Geosciences at the University of Texas at Austin. Water samples were analyzed for trace elements (^{88}Sr, ^{137}Ba, ^{55}Mn, ^{24}Mg, ^{40}Ca) using an Agilent 7500ce quadrupole inductively coupled plasma mass spectrometer (ICPMS) run in solution mode. Prior to analysis, samples were diluted by a factor of 10, 20, or 100x (depending on sample salinity) using 2% nitric acid to obtain less than 500 ppm total dissolved solids. Machine drift was compensated for by spiking selected samples with an internal standard solution. Mean recovery for spiked elements was 99%. Accuracy was calculated within 10% for all elements by using National Institute of Standards and Technology (NIST) 1643e as an external reference standard, diluted 10x.

Otoliths were analyzed using laser ablation – ICPMS using an Agilent 7500ce quadrupole ICPMS. Otoliths were loaded into the laser using a large-format laser cell that allowed multiple slides to be loaded into the chamber at once. Otolith runs were bracketed by two certified reference materials standards, NIST 612 (glass) and MACS3 (carbonate), every 15 analyses. Prior to ablation, otoliths were cleaned using a low-powered laser cleaning pulse, with a spot size of 50 µm, to remove a thin surface layer and any contamination. Otoliths were analyzed for ^{88}Sr, ^{137}Ba, ^{55}Mn, ^{24}Mg, ^{40}Ca. Otoliths were ablated from the core to the distal edge along the sulcal groove. A spot size of 35 µm with a scan speed of 3 µm/sec was used.

Elemental counts were converted to element:Ca ratios using the bracketed standard approach described by Rosenthal et al. (1999). Briefly, background elemental intensities were subtracted from all measurements, correction factors for elemental mass bias were calculated and linearly interpolated between adjacent analyses of the MACS3 standard, and finally precision was assessed by replicate measurements of the NIST 612 standard. Estimates of analytical precision (relative standard deviation) across all runs were (n = 38) was 5.5% for Sr/Ca and 10.5% for Ba/Ca.

Following LA-ICPMS analyses, the otoliths were photographed using an Olympus BX41 microscope with a TRITC filter under 20x magnification that allowed the ARS mark to become visible. Using Image J, accretion measurements were taken from the edge of the first ARS mark to second ARS mark and then to the edge of the otolith, parallel to the laser ablation transect.

Where no second ARS mark was present, measurements were made from the first ARS mark to the edge of the otolith. These measurements were then used to identify the experimental period in the otolith when examining the LA-ICPMS data.

Due to problems with the experiment (discussed below), I was unable to use otolith Ba/Ca values from during the experimental period to calculate D_{Ba}. However, D_{Ba} was calculated using the pre-experimental portion of the life history profile, during which all fish were reared continuously in fully marine water. The water value used to calculate the partition coefficient was an average of the marine water sampled in Chapter 1 and the marine water sampled in the experiment.

A one-way nested ANOVA with tanks nested within salinity treatments was conducted to determine if there were significant tank and treatment effects on otolith accretion for the phase one portion of the experiment.

Results

Unfortunately, there was significant mortality in all of the tanks that prevented the experiment from being completed successfully. While the majority of individuals survived through the end of experimental phase one, no individuals survived until the end of experimental phase two. Daily temperature and salinity data confirms that temperature remained relatively stable and salinity treatments were maintained for the duration of the experiment (Table 1.2). The mean water Ba/Ca in each tank throughout the experiment reveals that the Ba/Ca values were representative of the salinity regime, although Ba/Ca values in the fresh water treatment were more variable than those at 15 and 35 (Figure 2.1). Despite some variability in the water

Ba/Ca values, the salinity treatment levels appear distinct, supporting the fact that Ba/Ca is a robust proxy for ambient salinity. It is difficult to assess the Ba/Ca variability during phase two because in many cases, the fish only survived long enough for one water sample to be taken during phase two. Mean Ba/Ca values for each salinity treatment were comparable to the Ba/Ca values that are seen in the lower (estuarine) reaches of rivers along the south Texas Gulf Coast (Figure 2.2). Mean Ba/Ca experimental water values were lower across all three treatments as compared to the wild values, however, all of the mean experimental Ba/Ca water values fell within the variation of Ba/Ca values seen in local rivers.

Although Sr/Ca values appear to be less variable throughout the experiment as compared to Ba/Ca values, Sr/Ca values do not appear to be as sensitive to shifts in salinity (Figure 2.3). For example, the 0 salinity treatment is distinct, but the 15 and 35 salinity treatments do not appear to have distinctly different Sr/Ca values. This once again demonstrates that for this particular system, Sr/Ca values are not as useful at discriminating movement patterns across a salinity gradient as Ba/Ca values. As compared to the Sr/Ca values seen in rivers along the Texas Gulf Coast, the experimental Sr/Ca values are consistently lower, which is particularly evident at a salinity of 35 (Figure 2.4).

Somatic growth data indicates that there was very little fish growth throughout the experimental period (Table 2.3). Note that these growth measurements are calculated using mean initial and final TL of fish in each tank and are not based on individual growth measurements. Total growth ranged from -1.0 cm to 2.4 cm TL. Otolith growth was measured in the amount of otolith accretion that occurred during the first experimental period. Because there was significant mortality during phase two of the experiment, and because mortality events occurred at different times in different tanks, otolith accretion was only reported for the first phase of the experiment. Otolith accretion measurements reveal that accretion was highly variable between individuals and between tanks (Figure 2.5). Tank 18 had the highest mean accretion at 103.37 ($\pm$ 43.86) µm, while tank 5 had the lowest mean accretion with 29.79 ($\pm$ 9.19) µm. There was a significant interaction between salinity treatment and tank on accretion (p = 8.453 x 10^{-5}, Table 2.4), indicating response of accretion to salinity was variable among tanks, making the affect of salinity uninterpretable.

As expected, the majority of individuals that were held at a salinity of 35 throughout the experiment did not appear to have increases in the concentration of otolith Ba/Ca (Figure 2.6). However, there were some individuals where otolith Ba/Ca did deviate from this expected pattern (Figure 2.7). Individuals that were exposed to significant shifts in salinity did show rapid increases in otolith Ba/Ca within a few days (Figures 2.8, 2.9). Individuals that were held at salinities of 0 for the entirety of experiment did not appear to reach an equilibrium otolith Ba/Ca value (Figures 2.8, 2.9). Final otolith Ba/Ca values for these individuals held at salinities of 0 for both phase one and phase two varied widely, ranging from 30 to 100 µmol/mol. This variability most likely resulted from the extremely low growth and otolith accretion, making determination of final values difficult. These values are consistent with Ba/Ca values for individuals classified as using low salinity habitat in Chapter 1. Using the pre-experimental portion of the life history profile from marine rearing, the partition coefficient was calculated to be 0.04 $\pm$ 0.006 (SD)

Discussion

Due to extremely low growth rates and significant mortality events, I was unable to accomplish the objective of determining the otolith equilibration rates of Ba/Ca in juvenile southern flounder. There are several possibilities for why this experiment did not provide significant results. First, throughout the course of the experiment, fresh water was added to the 15 and 35 salinity tanks to maintain salinity, which could have contributed to some of the unexpectedly high Ba/Ca values seen in the otoliths of fish in these treatments. Secondly, it is possible that the dark tank color (black) and the heavy mesh on top of the tanks to prevent escape prevented individuals from exhibiting normal feeding behavior. During the experiment, fish were observed to be feeding minimally, supporting the conclusion that minimal growth hindered the experiment. Low growth makes the interpretation of otolith elemental data particularly difficult because a low growth rate means that a static laser spot diameter integrates more time than it would be if the fish was exhibiting a typical growth pattern, thereby precluding the ability to fully establish if equilibration was occurring. In future studies, using a lighter tank color and lighter mesh may encourage better feeding behavior.

Although previous studies have reported low mortality for the ARS treatment, it is possible that exposing these fish to the ARS treatment twice resulted in high stress, and therefore low growth and high mortality. ARS treatments are highly stressful for the fish not only because of the exposure to the ARS, but also because these treatments require starvation periods and a high level of handling. The experimental design also contributed to the uninterpretable results from the experiment. In this experiment I visually marked otoliths with ARS and later made measurements of the placement of the ARS band to evaluate the chemical profiles from each otolith. However, when using a laser spot that integrates material over a 35 μm region, it is difficult to separate the end of one phase from the beginning of another phase. Therefore, I recommend in future studies an otolith chemical signature (such as the stable isotope markers explored in Woodcock et al. 2011) be used. This way, the beginning and end of the experimental periods will be evident on the life history profiles and excising the portion of the otolith that accreted during each phase will be more precise. Using stable isotope markers could also decrease stress on the fish, since they do not require the stress of starvation and handling of the fish.

Although I cannot draw firm conclusions about equilibration from these results, looking at the otolith elemental profiles does reveal some interesting patterns, that although not conclusive, are still intriguing. Similar to the results found in Miller (2011), this experiment demonstrated that when individuals are exposed to a significant shift in salinity and elemental concentrations of Ba, the initial change in the otolith composition can occur within a few days. Fish in tanks 7 and 14 were held in the 0 salinity treatment for the duration of the experiment, yet otolith Ba/Ca values did not appear to reach equilibrium. However, even if equilibrium were reached it may not have been detectable given the extremely low growth and accretion that occurred. Accretion was less than 60 μm for the majority of fish, meaning that with a laser diameter of 35 μm, the analyses of the experimental period had limited resolution. Thus, even if equilibrated material was present in a relatively thin band, this signal would be swamped out by adjacent non-equilibrated material included in the ablation. The variable final Ba/Ca values are also not surprising given the highly variable accretion measurements. The fact that it could take juvenile southern flounder over 30 days to equilibrate an environmental signal is consistent with the results found in Yokouchi et al. (2011), which indicated that it could take anywhere from 30 – 60 days for a signal to become equilibrated. Additionally, in order to maintain salinities of 35 and 15 and to counteract evaporative effects, it was occasionally necessary to dilute the seawater treatment with fresh water, which could have altered the water chemistry in between water sampling events. These factors together help explain the sometimes unexpected patterns observed in the elemental profiles during the experimental period.

Despite the issues associated with the determination of equilibration rates, analyses of these fish allowed the calculation of the D_{Ba} partition coefficient. Because all fish had been reared in fully marine water for their entire lives prior to the onset of the experiment, elemental profiles of Ba/Ca were consistently low prior to the initial ARS mark. This coupled with the numerous measurements of marine Ba/Ca ratios allowed the D_{Ba} to be calculated with confidence.

The partition coefficient calculated and applied to these fish (0.04) is similar to partition coefficients calculated for a broad range of species. The Ba/Ca partition coefficients for other species range from 0.02 to 0.32 (as reviewed in Miller et al. 2009). Although the partition coefficient calculated in this study is on the lower end of this range, it does still fall within the range of previously calculated Ba/Ca partition coefficients. There are many different factors that affect the uptake and incorporation of elements into the otolith, so there will be some amount of error around the elemental values found in the otolith that are used to define habitat zones, which must be considered along with the variability in elemental concentrations between tributaries. However, the partition coefficients and threshold values used in this study are conservative, so I am confident in the interpretation of the patterns that were seen in the otoliths.

The partition coefficient calculated here is integral to interpreting elemental profiles of Ba/Ca in otoliths from wild fish. This value in combination with geographic assessments of estuarine and freshwater elemental compositions allows thresholds to be determined that indicate movement across salinity gradients. In the future, experiments that can successfully determine equilibration rates and partition coefficients across salinity gradients for southern flounder should be pursued to further disentangle the potential effects of salinity on elemental uptake and incorporation for this highly mobile species.

Chapter 2 Tables

Treatment		
Phase One	**Phase Two**	**Tanks**
0	0	7, 14
0	15	15
0	35	11, 12, 13
15	0	3, 4
15	15	10, 18
15	35	1, 8
35	0	5, 6, 17
35	15	
35	35	9, 16

Table 2.1. Experimental design. Due to mortality, tanks 1, 8, and 15 were not exposed to the phase two treatment. Originally, the experiment was designed to have two replicates of each treatment. Due to mortality, some of the phase two treatments were adjusted.

	Phase One		Phase Two	
Tank	**Temperature(°C)**	**Salinity**	**Temperature(°C)**	**Salinity**
1	19.6 ± 0.4	15.8 ± 0.6	NA	NA
2	19.6 ± 0.3	35.2 ± 1.1	NA	NA
3	19.7 ± 0.4	15.7 ± 1.2	19.7 ± 1.1	1.8 ±0.6
4	19.6 ± 0.3	16.1 ± 1.0	19.7 ± 1.2	1.70 ± 0.3
5	19.6 ± 0.3	35.0 ± 1.6	19.3 ± 2.2	2.80 ± 3.0
6	19.5 ± 0.3	35.8 ± 1.2	19.5 ± 1.2	1.90 ±1.4
7	19.6 ± 1.4	1.10 ± 0.34	18.7 ± 1.3	1.60 ± 0.1
8	19.4 ± 0.5	15.7 ± 0.8	NA	NA
9	19.5 ± 0.3	35.6 ± 1.0	18.7 ± 1.3	32.0 ± 0.7
10	19.6 ± 0.3	15.4 ± 2.7	18.6 ± 1.3	15.2 ± 0.7
11	19.8 ± 1.2	1.10 ± 0.2	18.6 ± 1.3	32.2 ± 0.9
12	19.5 ± 2.1	1.10 ± 0.3	19.2 ± 1.1	33.4 ± 1.3
13	19.7 ± 1.4	1.20 ± 0.3	19.7 ± 0.3	32.1 ± 0.5
14	19.7 ± 1.5	1.10 ± 0.3	19.6 ± 0.7	1.70 ± 0.6
15	19.6 ± 1.7	1.20 ± 0.4	NA	NA
16	19.4 ± 0.3	35.2 ± 1.5	19.7 ± 0.4	32.7 ± 1.0
17	19.1 ± 0.4	35.7 ± 1.2	19.7 ± 1.0	2.00 ± 1.1
18	19.2 ± 0.4	16.4 ± 0.9	19.5 ± 0.5	15.4 ± 0.6

Table 2.2. Mean ± SD temperature and salinity from each experimental tank for phases one and two.

Tank	Mean Beginning TL (cm)	Mean Ending TL (cm)	Mean Growth (cm)
1	10.4 ± 0.9	9.7 ± 0.6	-0.7
2	11.6 ± 1.3	12.8 ± 2.5	1.2
3	11.4 ± 1.6	12.8 ± 1.7	1.3
4	11.6 ± 1.4	10.6 ± 0.7	-1.0
5	10.7 ± 0.7	10.2 ± 1.0	-0.5
6	11.4 ± 1.7	12.4 ± 2.4	1.0
7	11.7 ± 1.2	11.8 ± 0.6	0.1
8	11.9 ± 1.0	NA	NA
9	12.0 ± 1.0	12.9 ± 0.9	0.9
10	11.6 ± 1.9	13.3 ± 1.5	1.7
11	10.7 ± 1.0	10.7 ± 1.1	0.1
12	11.2 ± 1.5	10.5 ± 0.7	-0.7
13	10.1 ± 0.1	11.9 ± 0.3	1.8
14	11.5 ± 1.7	12.0 ± 0.7	0.5
15	10.7 ± 1.0	10.5 ± 1.0	-0.2
16	11.3 ± 1.2	12.4 ± 0.4	1.0
17	11.1 ± 1.5	10.9 ± 0.9	-0.2
18	11.9 ± 0.7	14.3 ± 0.5	2.4

Table 2.3. Mean ± SD beginning TL, ending TL, and growth for experimental tank. This table illustrates the extremely low growth of fish during the experimental period.

	Df	Sum Sq	Mean Sq	F Value	P
Treatment	2	2599.8	1299.89	3.1461	0.04964
Treatment:Tank	14	22557.1	1611.22	3.8996	8.453 e-05
Residuals	65	26856.2	413.17		

Table 2.4. Two-factor nested ANOVA comparing the otolith accretion between treatments, with tank nested within treatment.

Chapter 2 Figures

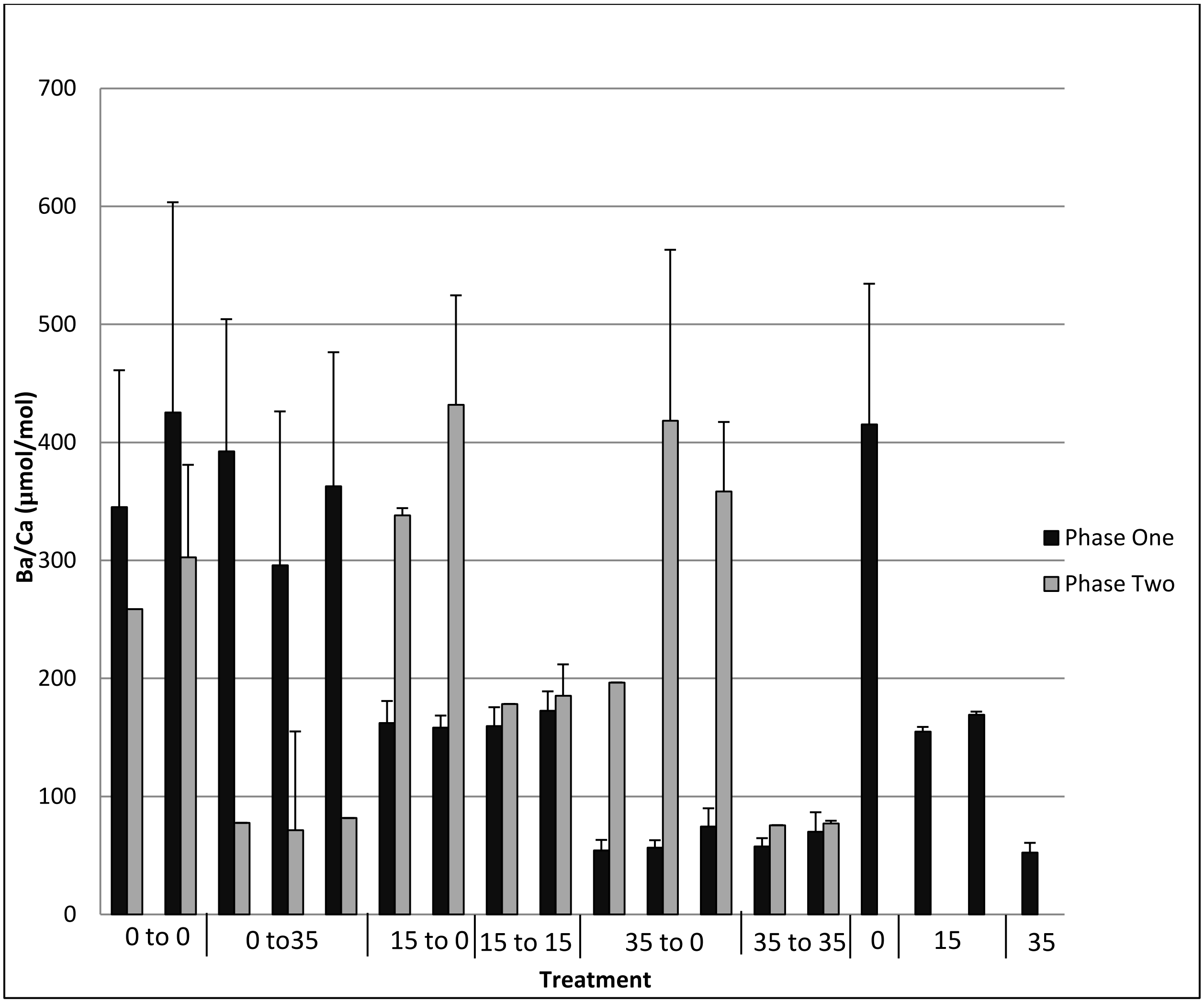


Figure 2.1. Mean ± SD water Ba/Ca (µmol/mol) plotted by treatment for phase one and phase two of the experiment. Salinity treatment for each phase is indicated by the lower label.

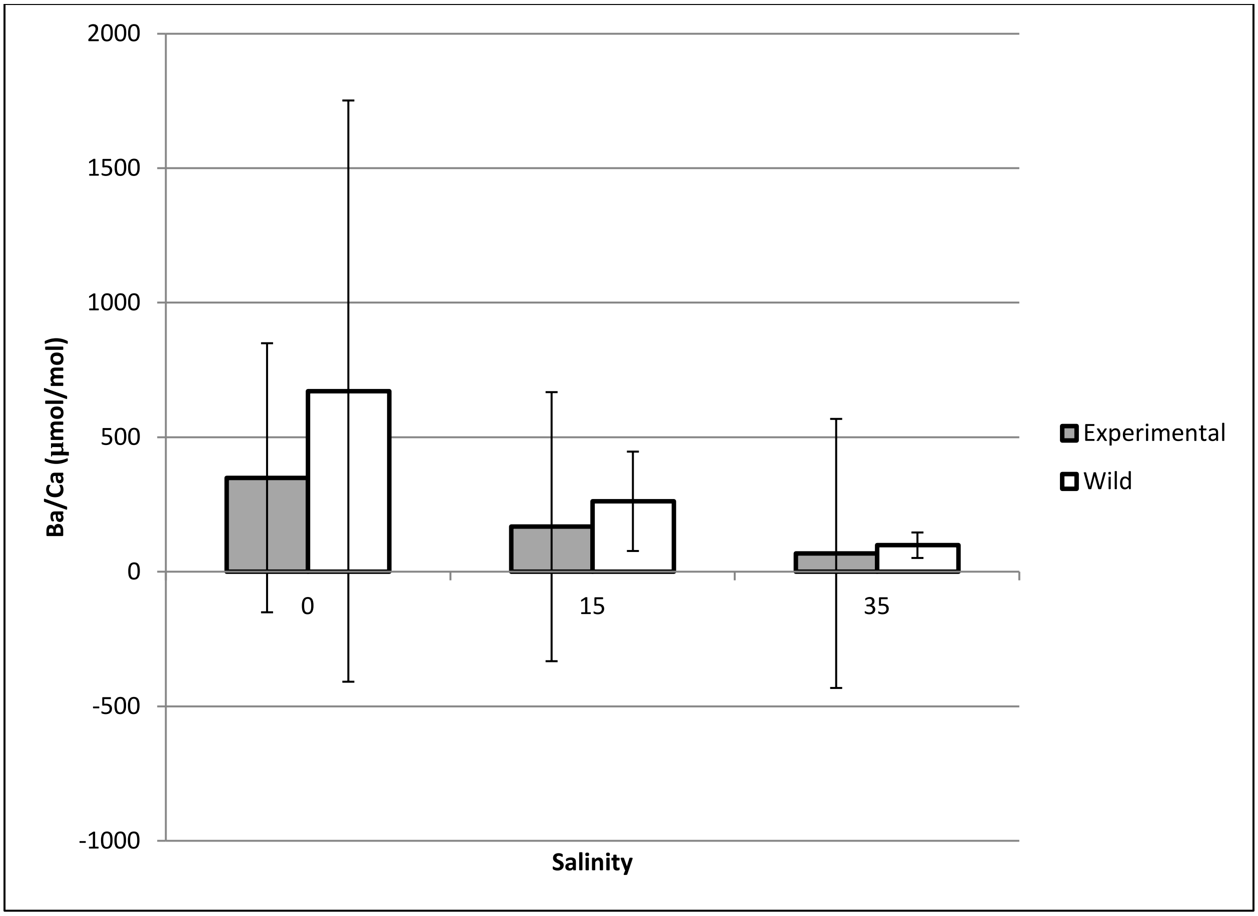


Figure 2.2. Mean ± SD experimental Ba/Ca (µmol/mol) for each salinity treatment plotted with the mean Ba/Ca values found at the same salinities in rivers along the south Texas Gulf Coast (wild data from Chapter 1).

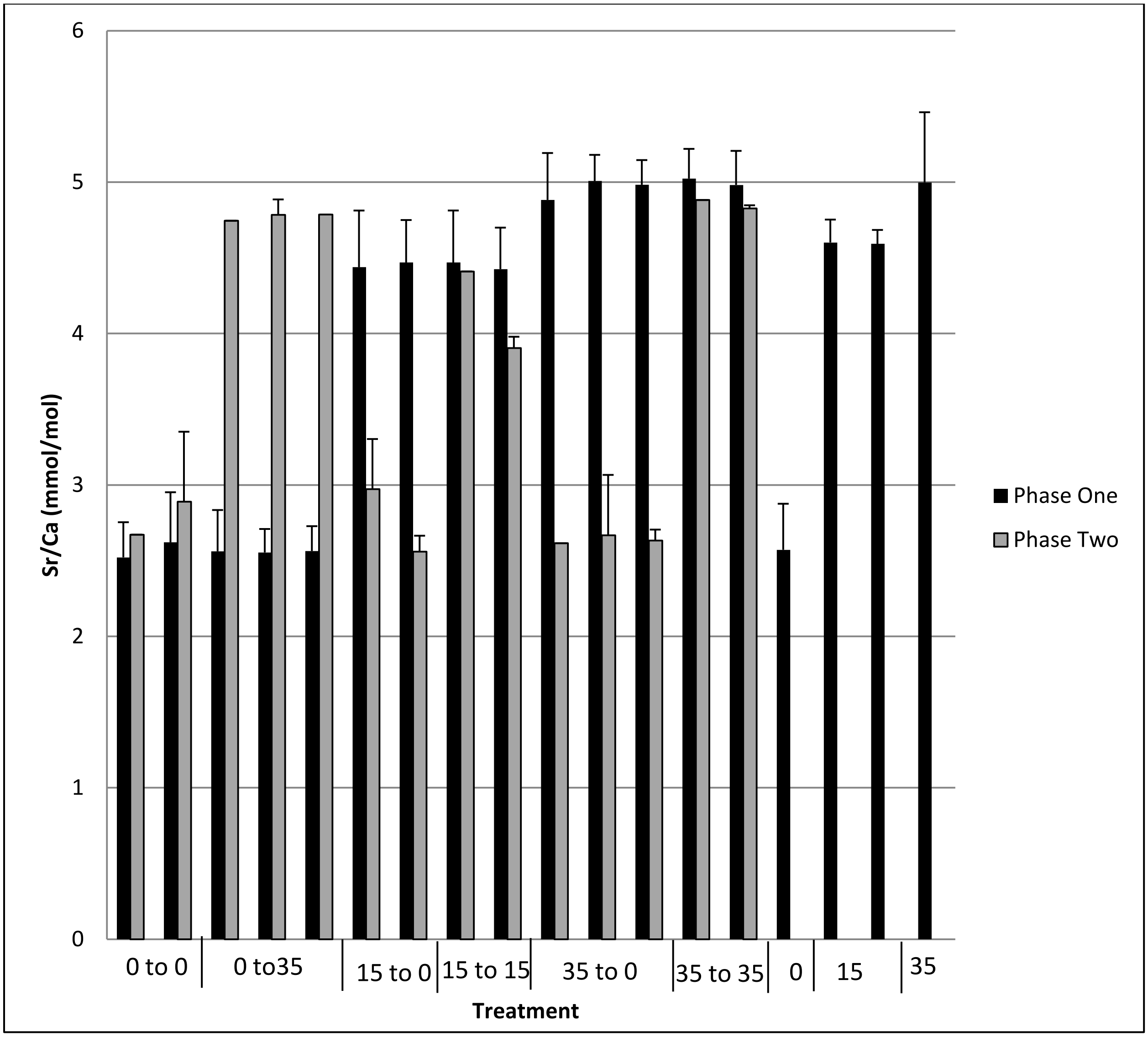


Figure 2.3. Mean water Sr/Ca (mmol/mol) by treatment for phase one and two of the experiment. Salinity treatment for each phase is indicated by the lower label.

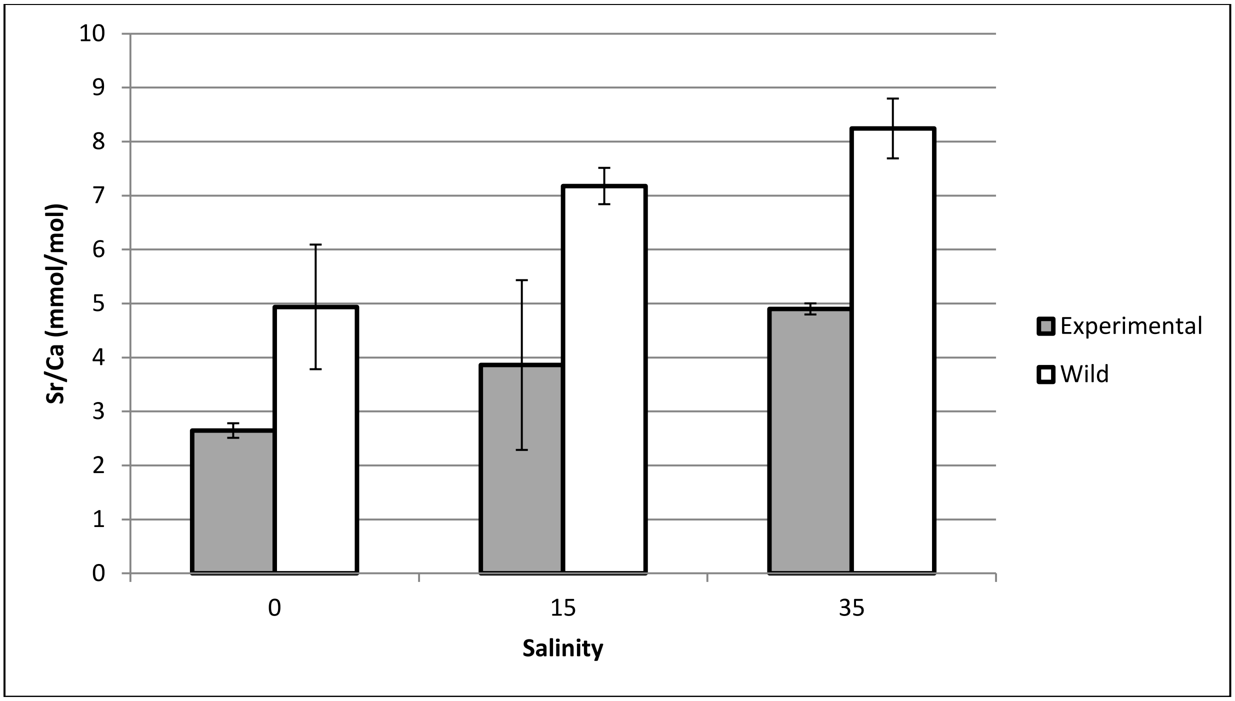


Figure 2.4. Mean ± SD experimental Sr/Ca (mmol/mol) for each salinity treatment plotted with the mean Sr/Ca values found at the same salinities in systems along the south Texas Gulf Coast (data from chapter 1).

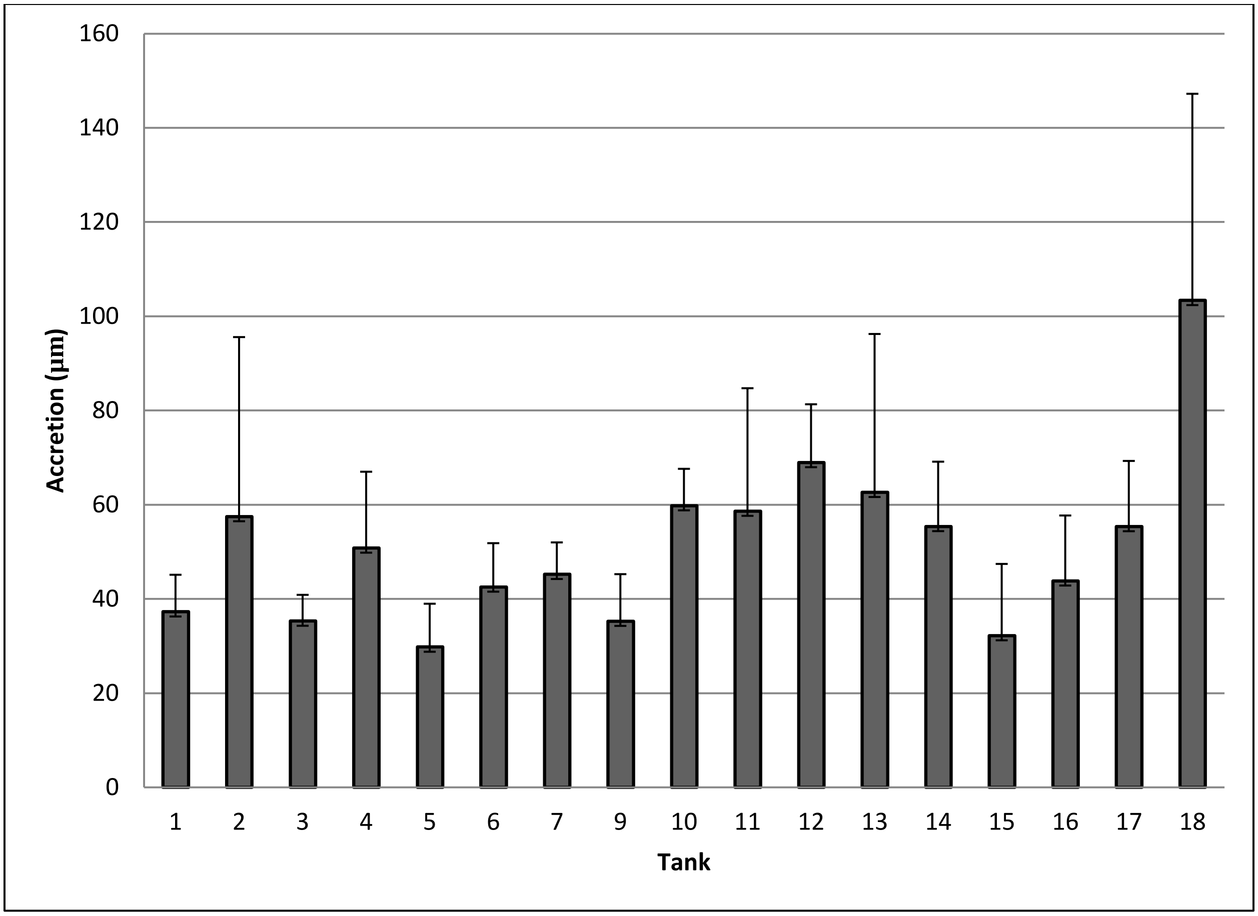


Figure 2.5. Mean ± SD otolith accretion by tank for the first phase of the experiment.

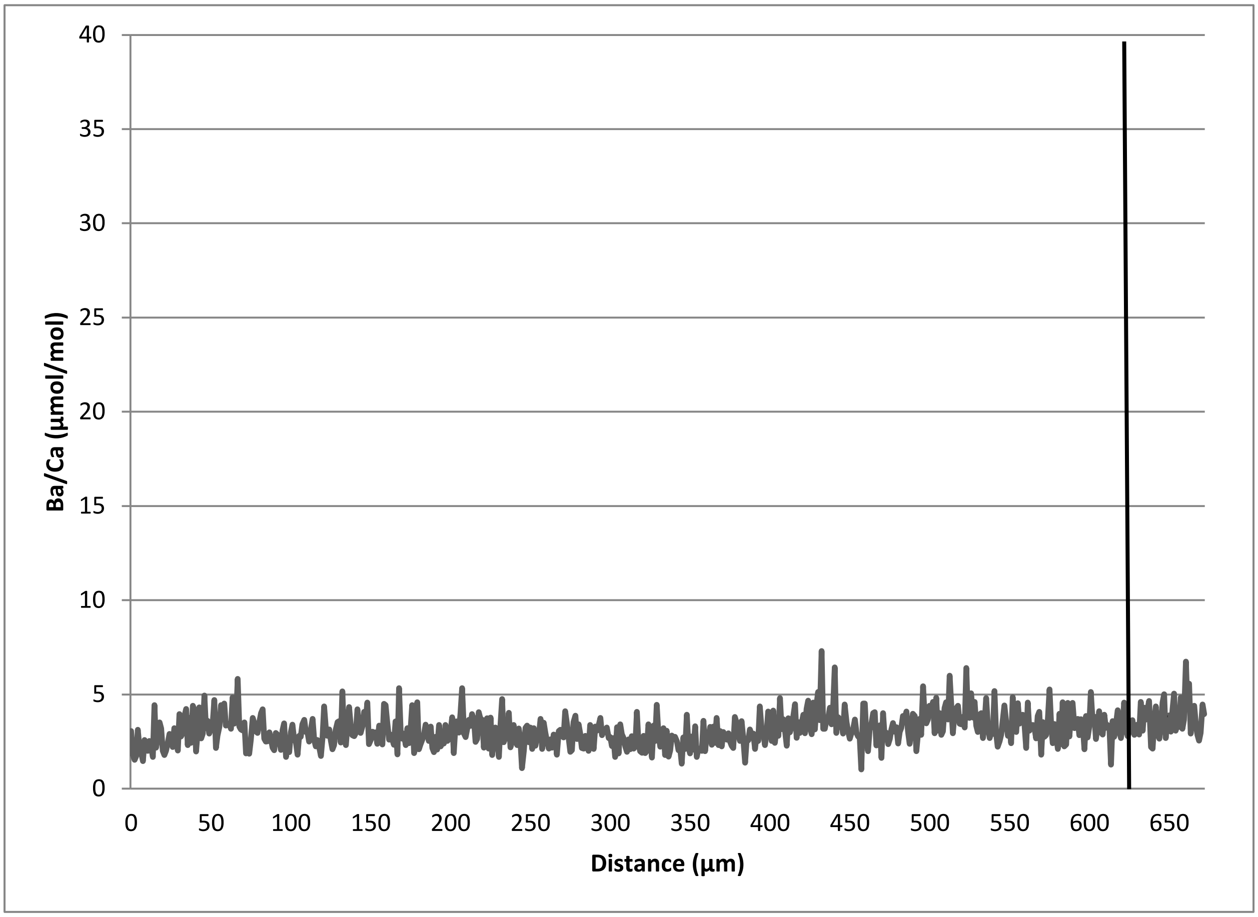


Figure 2.6. An example otolith life history profile for Ba/Ca from a fish in tank 1 that was exposed to 35 salinity water throughout the entire experiment. The black vertical line marks the beginning of the experimental period, as indicated by the presence of the ARS mark in the otolith. This individual exhibits the expected pattern of Ba/Ca when kept in 35 salinity water: the Ba/Ca value does not significantly change for the entirety for the fish's life.

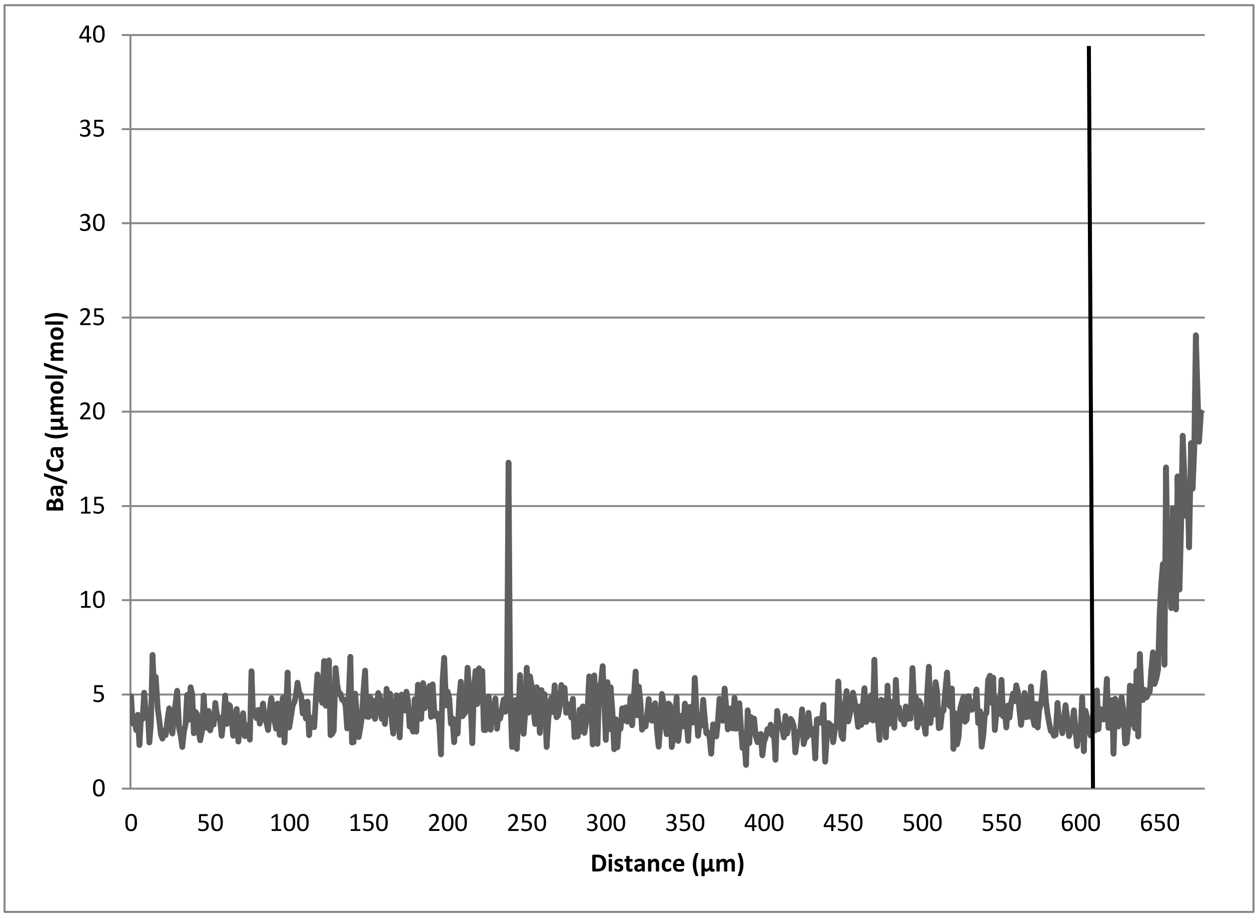


Figure 2.7. An example otolith life history profile for Ba/Ca from a fish in tank 1 that was exposed to 35 salinity water throughout the entire experiment. The black vertical line marks the beginning of the experimental period, as indicated by the presence of the ARS mark in the otolith. This individual exhibits an unexpected pattern of Ba/Ca when kept in 35 salinity water: the Ba/Ca value trends upwards towards the end of the fish's life.

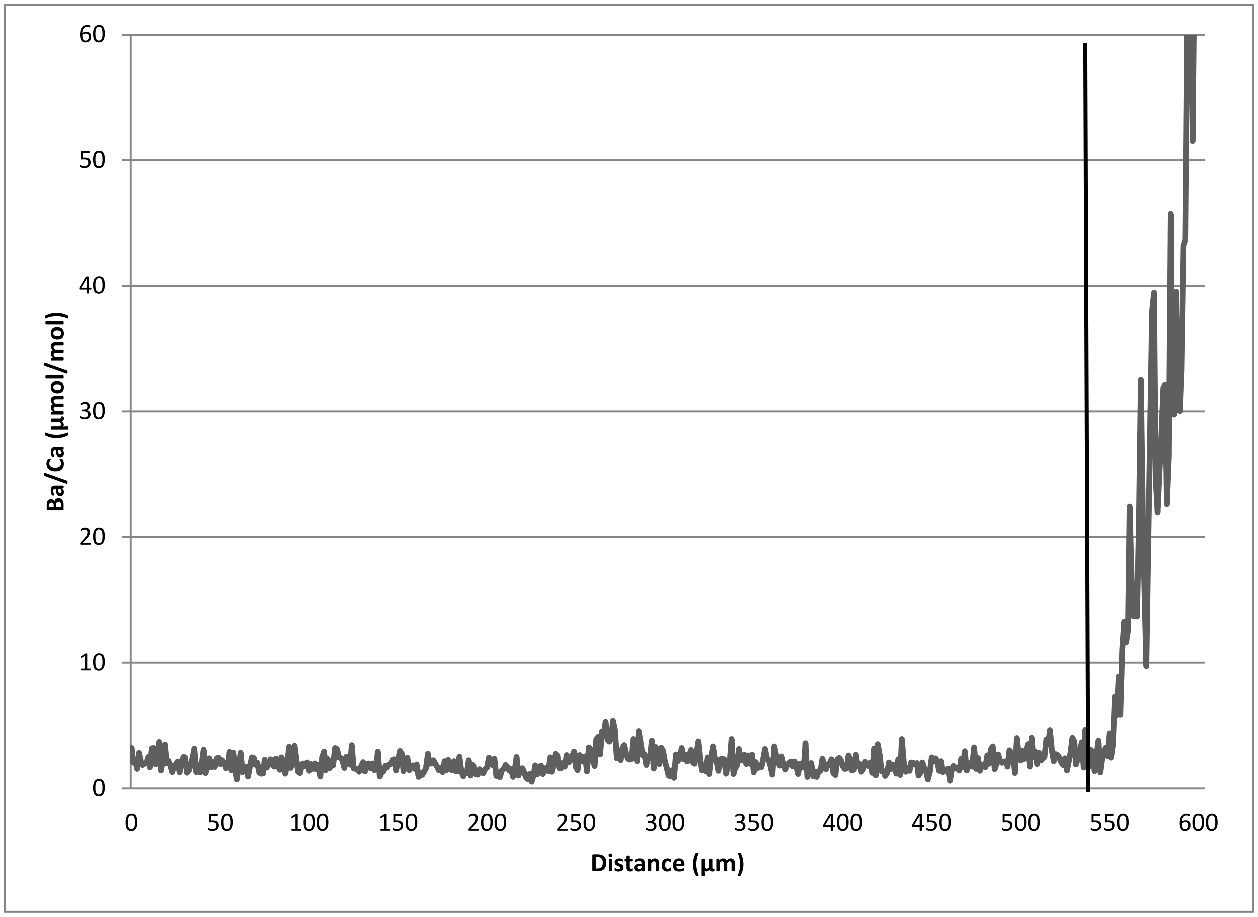


Figure 2.8. An example otolith life history profile for Ba/Ca from a fish in tank 7 that was exposed to 0 salinity water throughout the entire experiment. The black vertical line marks the beginning of the experimental period, as indicated by the presence of the ARS mark in the otolith. This individual exhibits an expected pattern of Ba/Ca when transferred from 35 to 0: Ba/Ca increases. This also shows the rapid change in Ba/Ca values when exposed to a significant shift in salinity. Ba/Ca values do not appear to reach equilibrium.

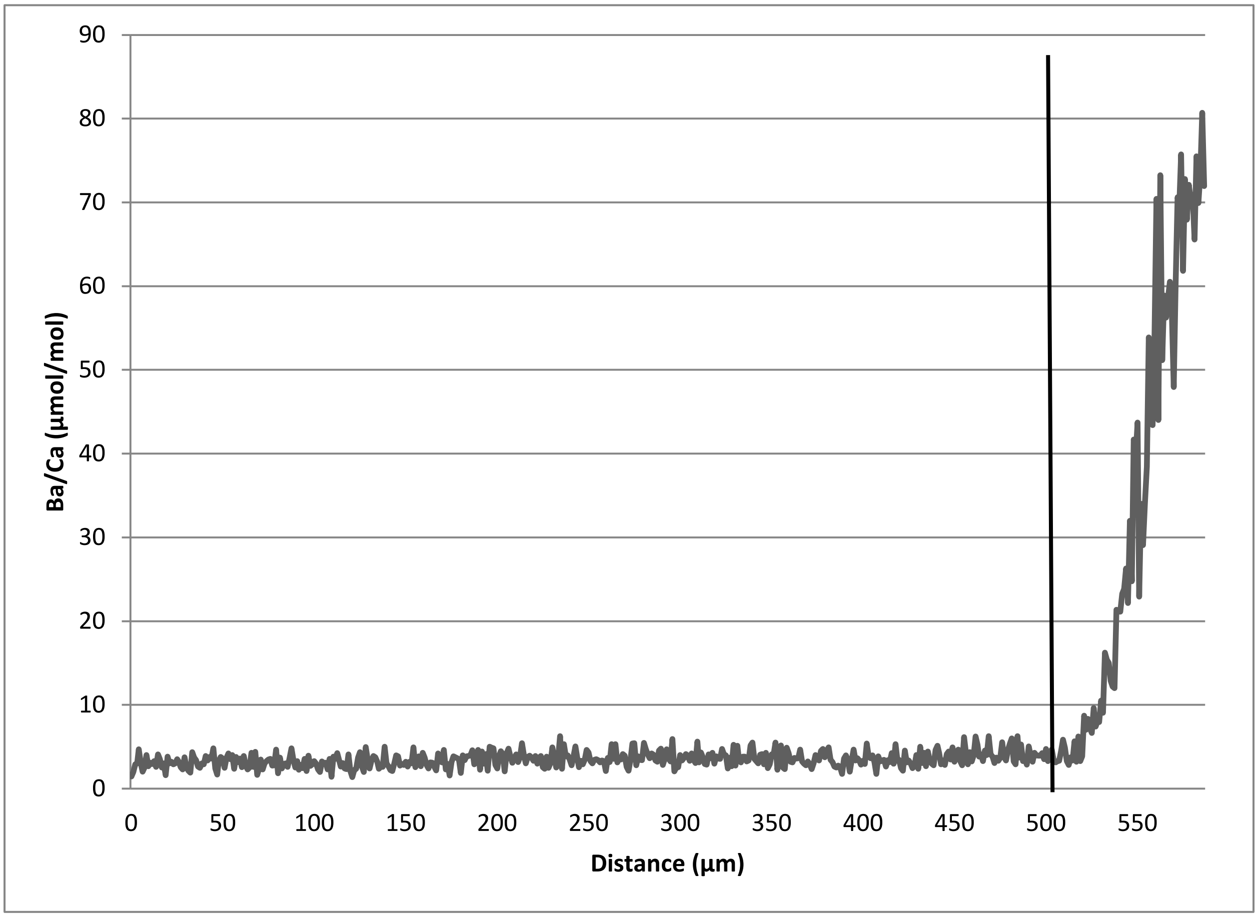


Figure 2.9. An example otolith life history profile for Ba/Ca from a fish in tank 14 that was exposed to 0 salinity water throughout the entire experiment. The black vertical line marks the beginning of the experimental period, as indicated by the presence of the ARS mark in the otolith. This individual exhibits an expected pattern of Ba/Ca when transferred from salinities of 35 to 0: Ba/Ca increases. This also shows the rapid change in Ba/Ca values when exposed to a significant shift in salinity. Ba/Ca values do not appear to reach equilibrium.

Appendix A: Water Data

Site	Salinity	Mg/Ca (mmol/mol)	Mn/Ca (mmol/mol)	Ba/Ca (mmol/mol)	Sr/Ca (mmol/mol)
NR 1	4.64	1295.3196	0.0661	0.3428	5.8174
NR 2	2.09	819.6299	0.0278	0.4303	5.3450
NR 3	1.14	484.3079	0.0280	0.5344	4.6104
NR 4	0.78	313.3894	0.1702	0.5779	4.5591
Guad 1	0.37	467.8420	0.0838	0.4033	4.2972
Guad 2	0.4	464.6788	0.0440	0.4064	4.3478
Guad 3	0.38	464.1262	0.0541	0.4042	4.3787
Guad 4	0.27	528.8903	0.0576	0.3757	4.4587
Guad 5	0.28	527.8584	0.0589	0.3829	4.4732
Oso 1	3.3	398.9791	0.0573	0.2120	5.6444
Oso 2	3.35	509.9366	0.2108	0.4091	5.9035
Oso 4	3.1	364.2928	0.1471	0.2001	5.6993
SA 1	0.73	394.3376	0.0382	0.2948	4.5857
SA 2	0.68	396.6049	0.0238	0.2866	4.6101
MR 1	1.34	894.7217	0.0676	1.1083	4.9619
MR 2	0.8	280.5942	0.5697	1.1386	5.7275
MR 3	1.42	909.7141	0.1152	1.1039	4.9985
Cavasso Crk	11.57	4251.7586	0.0323	0.3042	7.7474
AR 1	0.51	305.1926	0.0669	0.9480	2.6907
AR 2	0.62	277.6483	0.0643	0.9981	2.9705
Copano Bay	5.84	2858.4883	0.0723	0.7357	7.6370
SP 1	19.11	4473.3510	0.0095	0.0893	9.3152
SP 2	32.6	5095.0542	0.0170	0.0349	9.2710
AC 1	0.54	444.1362	0.0568	0.3822	7.0753
AC 2	0.5	411.6166	0.3417	0.3648	6.9818
NF	0.42	359.5277	0.1944	6.6630	6.6630

Table AA.1. 2010 elemental water ratios by site.

Site	Salinity (ppt)	Mg/Ca (mmol/mol)	Mn/Ca (mmol/mol)	Ba/Ca (mmol/mol)	Sr/Ca (mmol/mol)
AR 1	11.72	2839.4383	0.0086	0.6482	7.0146
AR2	1.34	510.0914	0.0246	0.7261	4.6536
RR	6.42	2856.2150	0.1024	0.5264	6.2749
COPANO BAY	34.11	5104.3485	0.3107	0.1781	8.1715
GUAD 1	4.83	2458.2138	1.0939	0.6070	6.0907
GUAD 2	0.55	580.0275	0.0324	0.7419	5.0467
GUAD 4	0.28	671.7851	0.1260	0.5104	4.9623
GUAD 5	0.36	79.5716	0.7418	0.2646	1.0454
VB CANAL	19.57	4230.6253	0.0635	0.2658	7.2767
MR 1	28.20	194.2075	0.5626	-0.0831	0.4735
MR 2	5.68	434.3747	0.3293	0.9434	11.5941
MR FEN RANCH	8.72	2449.5309	0.0422	0.8842	7.6718
NR 1	3.61	1607.7726	0.2622	0.4851	5.3738
NR2 R1	7.94	2198.3161	0.0505	0.2920	6.1601
NR2 R2	7.94	2181.6184	0.0385	0.2997	6.1198
NR3	13.99	2858.9342	0.0409	0.2018	6.8818
NR4 R1	17.96	3203.1232	0.0307	0.1636	7.0610
NR4 R2	17.96	3249.3902	0.0404	0.1599	7.1757
NR5	23.27	3629.0641	0.0196	0.1392	7.3150
NR6 R1	28.58	3849.3405	0.0237	0.1309	7.5439
NR6 R2	28.58	3838.7106	0.0242	0.1282	7.5804
NR 7	33.22	4168.6786	0.0228	0.1094	7.7351
NR 8 R1	38.82	4396.0176	0.0141	0.0989	7.9045
NR 8 R2	38.82	4472.3376	0.0197	0.0988	7.9707
NR 9	49.96	5150.6875	0.0677	0.0717	8.4029
NR10 R1	0.72	386.0078	0.0311	0.6385	4.1734
NR10 R2	0.72	385.9932	0.0344	0.6356	4.2028
OSO 1	2.48	405.1349	0.0754	0.1466	5.5327
OSO 2	2.19	480.7765	0.0500	0.1740	5.0621
OSO 4	2.73	389.0924	0.5053	0.1309	5.5491
SA 1	0.92	453.8693	0.1193	0.2783	5.5805
SA2	0.90	457.9532	0.0669	0.1920	4.7157

Table AA.2. 2011 water elemental ratio data by site.

Appendix B: Supplemental Otolith Data

Fish ID	Location	Date Captured	TL (cm)
1	Ship Channel	2007-2009	30.6
2	Ship Channel	2007-2009	32
3	Ship Channel	2007-2009	28.2
4	Ship Channel	2007-2009	33.6
6	Ship Channel	2007-2009	29.8
7	Lydia Ann	3/22/2011	25.72
8	Lighthouse Lakes	1/6/2011	35.56
9	Lighthouse Lakes	1/6/2011	38.1
10	Lighthouse Lakes	1/6/2011	34.6
11	Lighthouse Lakes	1/6/2011	33.3
12	Lighthouse Lakes	1/6/2011	35.56
13	Lighthouse Lakes	1/21/2011	24.45
14	Lighthouse Lakes	1/6/2011	22.86
15	Lighthouse Lakes	1/21/2011	35.24
16	Ship Channel	2011	57
17	Ship Channel	2011	52.7
18	Ship Channel	Fall 2010	59.1
19	Ship Channel	Fall 2010	51
20	Ship Channel	fall 08 or 09	42.2
21	Ship Channel	2010	54.6
22	Ship Channel	2008/2009	40
29	Redfish Bay	4/14/2011	3.75
30	Redfish Bay	4/15/2011	3
31	Ship Channel	2009	44
32	Ship Channel	2009	42.5
33	Ship Channel	2009	32
34	Ship Channel	2009	26.5
35	Ship Channel	2009	32.3
37	Ship Channel	2009	54
38	Ship Channel	2010	34.5
39	Ship Channel	2010	40.3
40	Ship Channel	2010	33.1
41	Ship Channel	2010	35.2
42	Ship Channel	2010	32.6
43	Ship Channel		40.3
44	Ship Channel	2010	32.9
45	Ship Channel		32.9

46	Lydia Ann	2011	44.8
47	Lydia Ann	2011	40
48	Lydia Ann	2011	39
49	Lydia Ann	2011	42.5
50	Lydia Ann	2011	39.9
51	Lydia Ann	2011	33.2
52	Lydia Ann	2011	44.7
53	Lydia Ann	2011	36.5
54	Lydia Ann	2011	32.5
55	Lydia Ann	2011	35.1
56	Lydia Ann	2011	39.5
57	Lydia Ann	2011	40
58	Lydia Ann	2011	35.9
59	Lydia Ann	2011	36
60	Lydia Ann	6/17/2011	17.5
61	Lydia Ann	6/17/2011	18
62	Ship Channel	2009-2010	43.5
63	Ship Channel	2009-2010	44.1
64	Ship Channel	2009-2010	40
65	Ship Channel	2009-2010	34.8
66	Ship Channel	2009-2010	37.5
67	Ship Channel	2009-2010	37
68	Ship Channel	2009-2010	40.1
69	Ship Channel	2009-2010	42
70	Ship Channel	2009-2010	46
71	Ship Channel	2009-2010	43.5
72	Ship Channel	2009-2010	40
73	Ship Channel	2009-2010	28.5
74	Lydia Ann	12/14/2009	36.2
75	Lydia Ann	12/14/2009	38.4
76	Lydia Ann	12/14/2009	38.5
77	Lydia Ann	12/14/2009	43
78	Lydia Ann	12/14/2009	41.9
79	Lydia Ann	12/14/2009	42
82	Lydia Ann	12/14/2009	50.7
83	Lydia Ann	12/14/2009	58.1
84	Lydia Ann	12/14/2009	46.9
87	Ship Channel/Lydia Ann	Fall 2009/2010	38.2
88	Ship Channel/Lydia Ann	Fall 2009/2010	37

89	Ship Channel/Lydia Ann	Fall 2009/2010	36.3
90	Ship Channel/Lydia Ann	Fall 2009/2010	45
91	Ship Channel/Lydia Ann	Fall 2009/2010	46.5
92	Ship Channel/Lydia Ann	Fall 2009/2010	39.5
93	Ship Channel/Lydia Ann	Fall 2009/2010	55
94	Ship Channel/Lydia Ann	Fall 2009/2010	30.7
95	Ship Channel/Lydia Ann	Fall 2009/2010	33.2
96	Ship Channel/Lydia Ann	Fall 2009/2010	37.1
97	Ship Channel/Lydia Ann	Fall 2009/2010	38.2
98	Ship Channel/Lydia Ann	Fall 2009/2010	40
99	Ship Channel/Lydia Ann	Fall 2009/2010	57.5
100	Rockport	7/11/2011	21.5
102	Lydia Ann	7/23/2011	29.7
103	Lydia Ann	7/24/2011	22
104	Lydia Ann	7/25/2011	20.4
105	Lydia Ann	7/26/2011	21.2
106	Lydia Ann	7/27/2011	17.3
110	Lydia Ann	11/28/2011	52.7
111	Lydia Ann	11/28/2011	41.5
112	Lydia Ann	11/28/2011	44.5
113	Lydia Ann	11/28/2011	51.5
114	Lydia Ann	11/28/2011	31.5
115	Lydia Ann	11/28/2011	42.5
116	Lydia Ann	11/28/2011	46.5
117	Lydia Ann	11/28/2011	36.8
118	Lydia Ann	11/28/2011	51.5
119	Lydia Ann	11/28/2011	42.5
120	Lydia Ann	11/28/2011	48.1
121	Lydia Ann	11/28/2011	49
122	Lydia Ann	11/28/2011	46.8
123	Lydia Ann	11/28/2011	46
124	Lydia Ann	11/28/2011	47.3
125	Lydia Ann	11/28/2011	49.8
126	Lydia Ann	11/28/2011	35.1
127	Lydia Ann	11/28/2011	34
128	Lydia Ann	11/28/2011	40.1
129	Lydia Ann	11/28/2011	41
130	Lydia Ann	11/22/2011	48.2
131	Lydia Ann	11/22/2011	29

132	Lydia Ann	11/22/2011	45.5
133	Lydia Ann	11/22/2011	37.8
134	Lydia Ann	11/22/2011	28.2
135	Lydia Ann	11/22/2011	30.5
136	Lydia Ann	11/22/2011	40.8
137	Lydia Ann	11/22/2011	44.2
138	Lydia Ann	11/22/2011	38.5
139	Lydia Ann	11/22/2011	47.1
140	Lydia Ann	11/22/2011	47
141	Lydia Ann	11/22/2011	49.5
142	Ship Channel	11/22/2011	42.3
143	Lydia Ann	11/22/2011	53.1
144	Lydia Ann	11/22/2011	42.5
145	Lydia Ann	11/22/2011	44.8
146	Lydia Ann	11/22/2011	45.9
147	Lydia Ann	11/22/2011	41.7
148	Lydia Ann	11/22/2011	49.8
149	Lydia Ann	11/22/2011	41
150	Lydia Ann	11/22/2011	42.9
151	Lydia Ann	11/22/2011	42.5
152	Lydia Ann	11/22/2011	47.2
153	Lydia Ann	11/22/2011	53
154	Lydia Ann	11/22/2011	35.5
155	Lydia Ann	11/22/2011	40
156	Lydia Ann	11/22/2011	41.9
157	Lydia Ann	11/22/2011	51.8
158	Lydia Ann	11/22/2011	43
159	Lydia Ann	11/22/2011	47.9
160	Lydia Ann	11/22/2011	51.7
161	Lydia Ann	11/22/2011	46.7
175	Sabine Lake	2011	39.8
176	Sabine Lake	2011	42.5
177	Sabine Lake	2011	42.5
178	Sabine Lake	2011	27.3
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180	Sabine Lake	2011	43.6
181	Sabine Lake	2011	51.6
182	Sabine Lake	2011	25
183	Sabine Lake	2011	26

184	Lydia Ann	2011	44.6
185	Lydia Ann	2011	47.5
186	Lydia Ann	2011	46.9
187	Lydia Ann	2011	44.2
188	Lydia Ann	2011	43
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192	Lydia Ann	2011	47
193	Lydia Ann	2011	53
194	Lydia Ann	2011	40.5
195	Lydia Ann	2011	34.5
196	Lydia Ann	2011	38
197	Lydia Ann	2011	34.4
198	Lydia Ann	2011	25
199	Lydia Ann	2011	44.5
200	Lydia Ann	2011	43
201	Lydia Ann	2011	47
202	Lydia Ann	2011	46
203	Sabine Lake	2011	45.5
204	Sabine Lake	2011	36.6
205	Lydia Ann	2011	49
206	Lydia Ann	2011	38
207	Lydia Ann	2011	36.5
208	Lydia Ann	2011	36
209	Lydia Ann	2011	42.5
210	Lydia Ann	2011	49.1
211	Lydia Ann	2011	41.9
212	Lydia Ann	2011	41
213	Lydia Ann	2011	45.5
214	Lydia Ann	2011	44
215	Lydia Ann	2011	44.3
216	Lydia Ann	2011	43
217	Lydia Ann	2011	32
218	Lydia Ann	2011	39.8
219	Lydia Ann	2011	35.3
220	Lydia Ann	2011	40.5
221	Lydia Ann	2011	44.1
222	Lydia Ann	2011	43.5

223	Lydia Ann	2011	45
224	Lydia Ann	2011	43.1
225	Lydia Ann	2011	47.2
226	Lydia Ann	2011	46.8
227	Lydia Ann	2011	51.2
228	Lydia Ann	2011	40.2
229	Lydia Ann	2011	53.2
230	Lydia Ann	2011	39
231	Lydia Ann	2011	38.7
232	Lydia Ann	2011	42.5
233	Lydia Ann	2011	50
234	Lydia Ann	2011	36.5
235	Lydia Ann	2011	49.9
236	Lydia Ann	2011	48.4
237	Lydia Ann	2011	48.5
238	Lydia Ann	2011	41
239	Lydia Ann	2011	42
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403	Lydia Ann	2011	48.3
404	Lydia Ann	2011	41.5
405	Lydia Ann	2011	46
406	Lydia Ann	2011	44
407	Lydia Ann	2011	43.5
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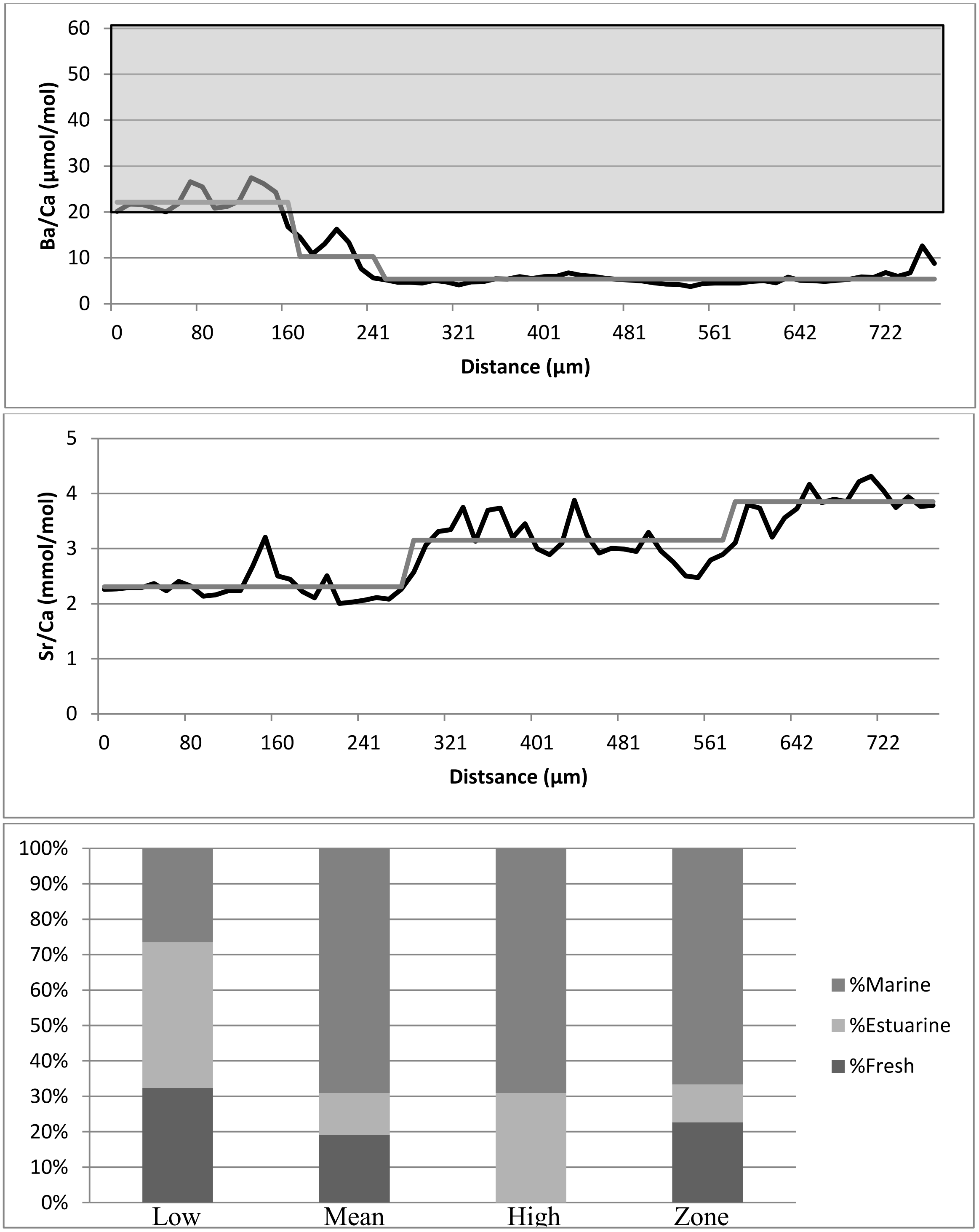
410	Lydia Ann	2011	42.5
411	Lydia Ann	2011	34
412	Lydia Ann	2011	38.8
413	Lydia Ann	2011	46
414	Lydia Ann	2011	47.3
415	Lydia Ann	2011	49.8
416	Lydia Ann	2011	68
417	Lydia Ann	2011	49.4
418	Lydia Ann	2011	47.8
419	Lydia Ann	2011	51.5
420	Lydia Ann	2011	41.6
421	Lydia Ann	2011	50.5

422	Lydia Ann	2011	40.5
423	Lydia Ann	2011	49
424	Lydia Ann	2011	35.5
425	Lydia Ann	2011	40.9
426	Lydia Ann	2011	41
427	Lydia Ann	2011	43.8
428	Lydia Ann	2011	40.5
429	Lydia Ann	2011	40
430	Lydia Ann	2011	42.8
431	Lydia Ann	2011	37.4
432	Lydia Ann	2011	37.4
433	Lydia Ann	2011	50.5
434	Lydia Ann	2011	42.9
435	Lydia Ann	2011	55.5
436	Lydia Ann	2011	42
437	Lydia Ann	2011	47
438	Lydia Ann	2011	42.5
439	Lydia Ann	2011	38.5
440	Lydia Ann	2011	44
441	Lydia Ann	2011	40
442	Lydia Ann	2011	43.5
443	Lydia Ann	2011	42.8
444	Lydia Ann	2011	38.1
445	Lydia Ann	2011	38.2
446	Lydia Ann	2011	36.6
447	Lydia Ann	2011	43.5
448	Lydia Ann	2011	43.5
449	Lydia Ann	2011	49
450	Lydia Ann	2011	37.9
451	Lydia Ann	2011	48
452	Lydia Ann	2011	50.8
453	Lydia Ann	2011	38.9
454	Lydia Ann	2011	40.2
455	Lydia Ann	2011	38.8
456	Lydia Ann	2011	38.5
457	Lydia Ann	2011	25.5
458	Lydia Ann	2011	39.5
459	Lydia Ann	2011	41.5
460	Lydia Ann	2011	48

461	Lydia Ann	2011	52.5
462	Lydia Ann	2011	42.5
463	Lydia Ann	2011	36
464	Lydia Ann	2011	46
465	Lydia Ann	2011	49.2
466	Lydia Ann	2011	21
467	Lydia Ann	2011	34.1
468	Lydia Ann	2011	35.9
500	Lydia Ann	2011	44
501	Lydia Ann	2011	46.5
502	Lydia Ann	2011	54
503	Lydia Ann	2011	43.9
504	Lydia Ann	2011	53.4
505	Lydia Ann	2011	40.6
506	Lydia Ann	2011	56
507	Lydia Ann	2011	52.8
508	Lydia Ann	2011	43.2
509	Lydia Ann	2011	40.4
510	Lydia Ann	2011	55.4
511	Lydia Ann	2011	40.6
512	Lydia Ann	2011	43.4
513	Lydia Ann	2011	41.6
514	Lydia Ann	2011	40.4
515	Lydia Ann	2011	32.9
516	Lydia Ann	2011	40.7
517	Lydia Ann	2011	29
518	Lydia Ann	2011	44.5
519	Lydia Ann	2011	47.5
520	Lydia Ann	2011	55
521	Lydia Ann	2011	51.9
522	Lydia Ann	2011	50.6
523	Lydia Ann	2011	55.6
524	Lydia Ann	2011	54.1
525	Lydia Ann	2011	51.5
526	Lydia Ann	2011	37.8
527	Lydia Ann	2011	51.8
528	Lydia Ann	2011	42.7
529	Lydia Ann	2011	49.8
530	Lydia Ann	2011	44

531	Lydia Ann	2011	54.8
532	Lydia Ann	2011	40.2
533	Lydia Ann	2011	56.2
534	Lydia Ann	2011	49
535	Lydia Ann	2011	44
536	Lydia Ann	2011	44
537	Lydia Ann	2011	53.5
538	Lydia Ann	2011	40
539	Lydia Ann	2011	53.2
540	Lydia Ann	2011	42.5
541	Lydia Ann	2011	40.5
542	Lydia Ann	2011	48.9
543	Lydia Ann	2011	47.6
544	Lydia Ann	2011	48.7

Table AB.1. Identification number, capture location, capture date, and total length (TL) for all wild fish captured in this study.



A.
B.
C.

Figure AB.1. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 1. Figure 1.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

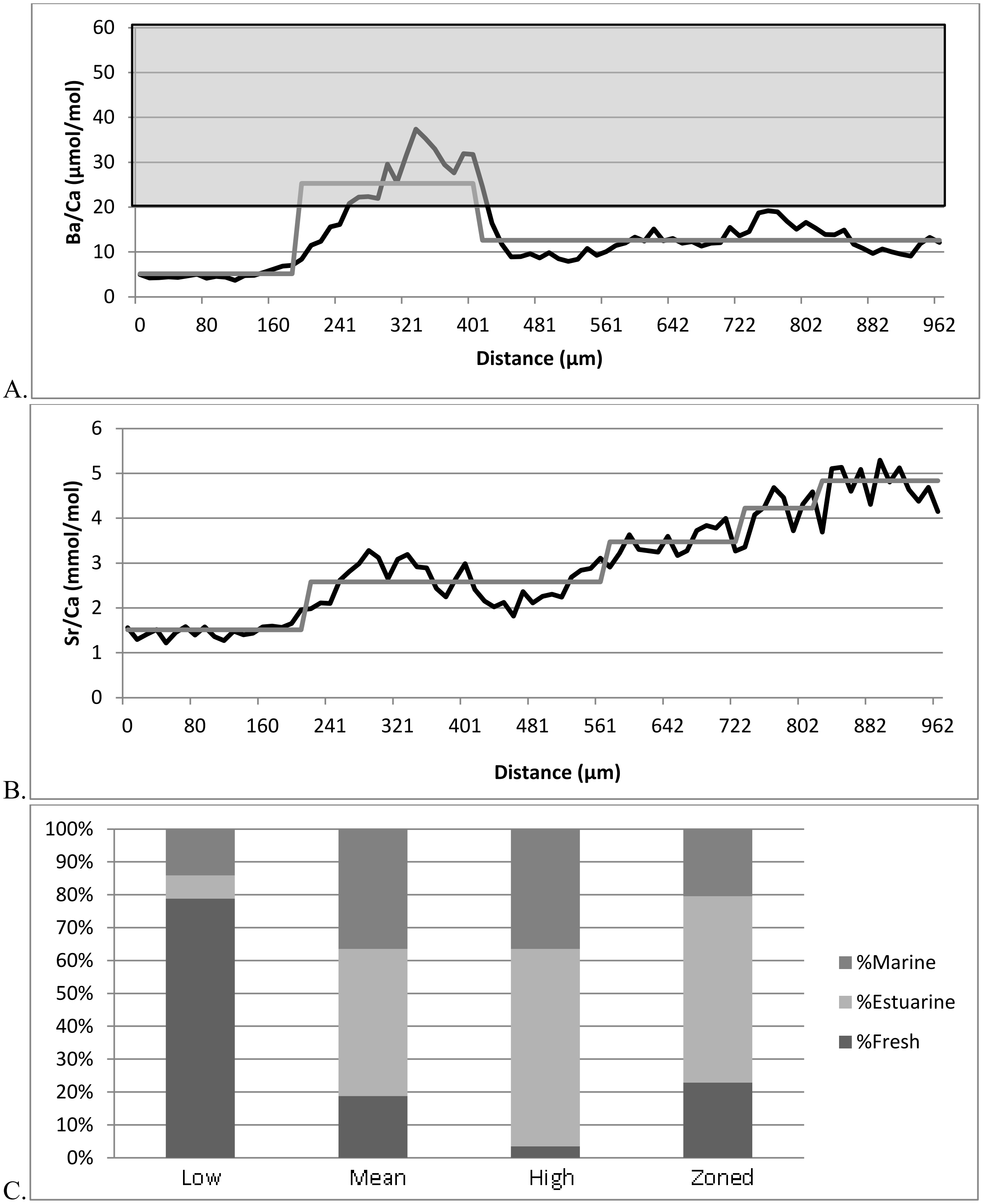
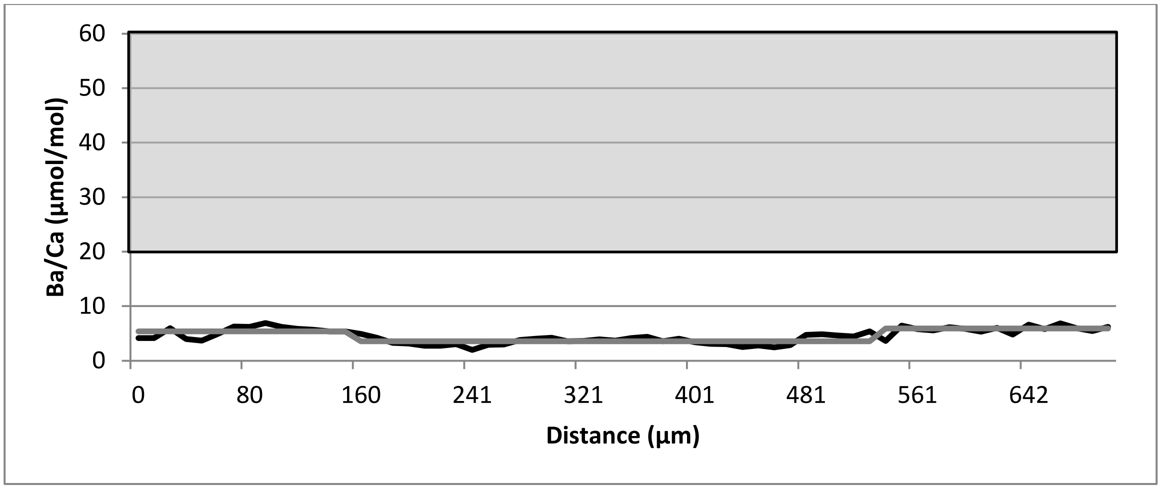
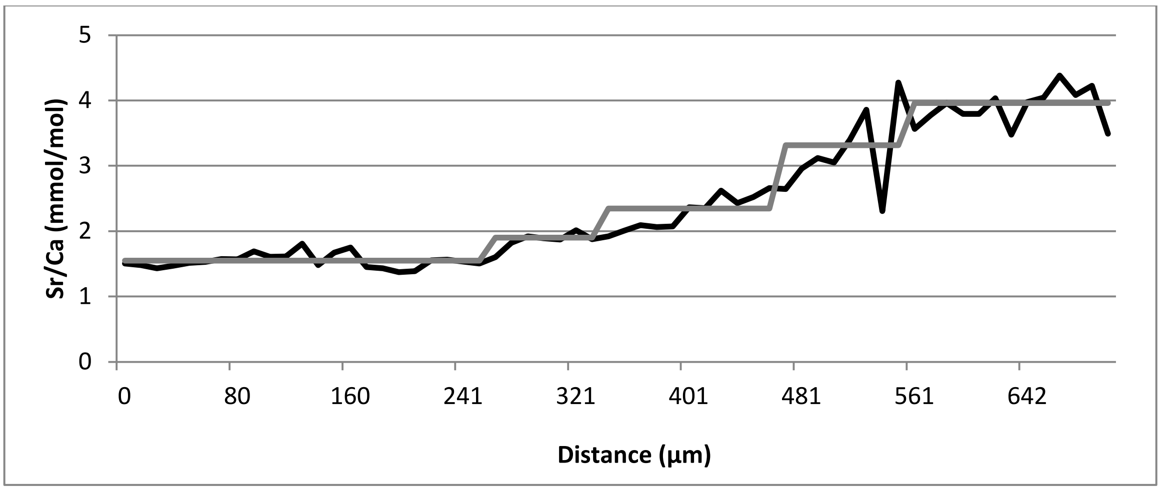


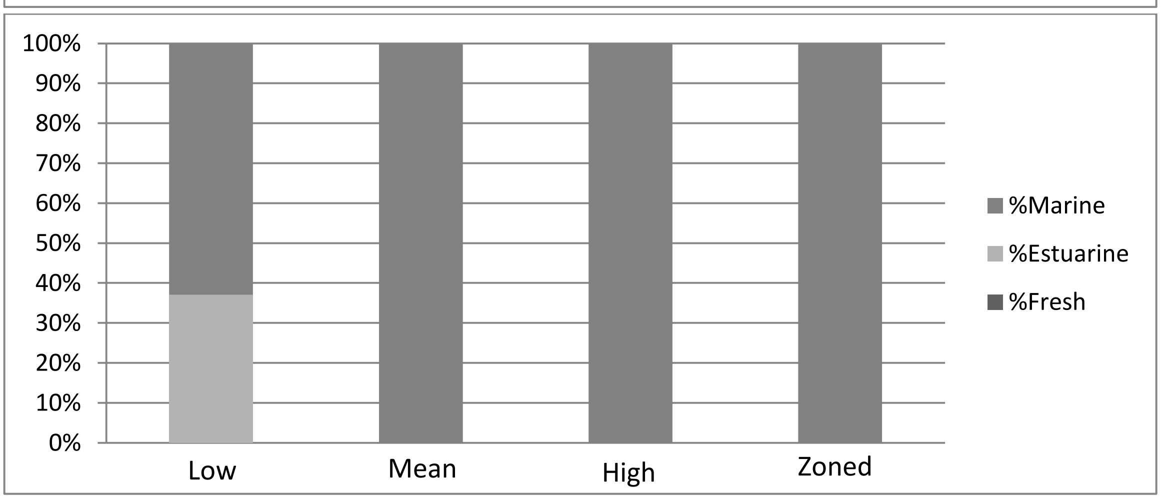
Figure AB.2. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 2. Figure 2.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

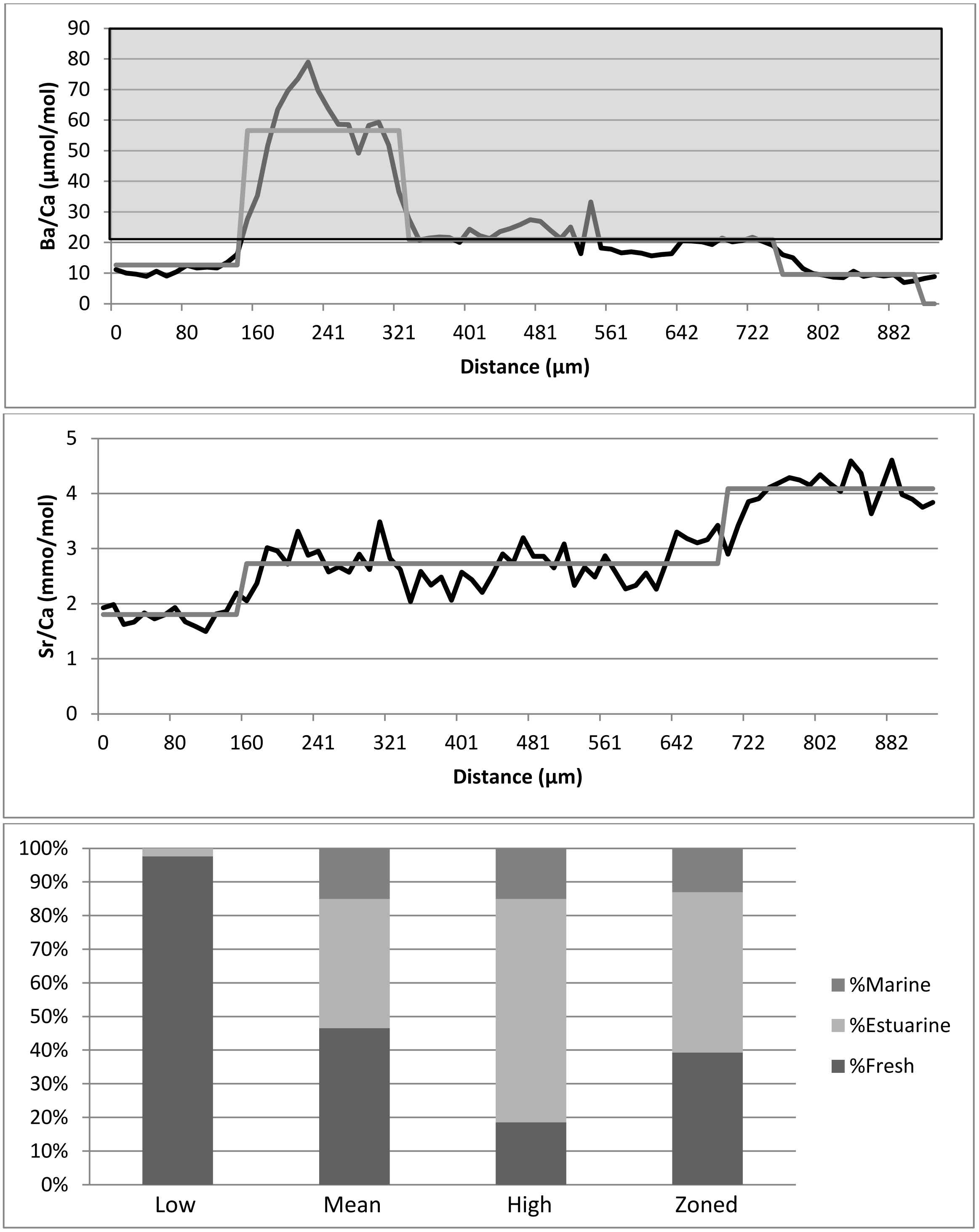


B.



C.

Figure AB.3. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 3. Figure 3.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

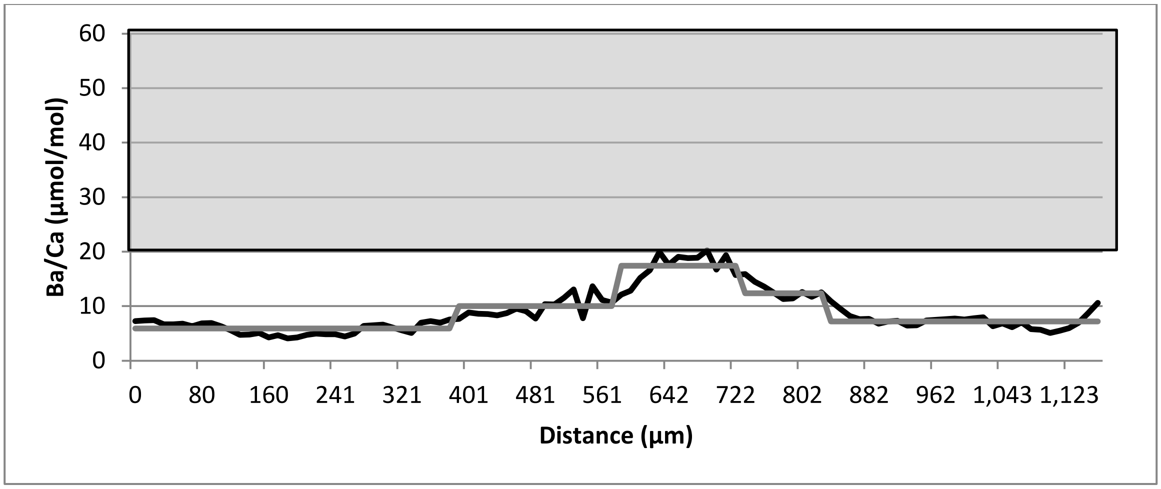


A.

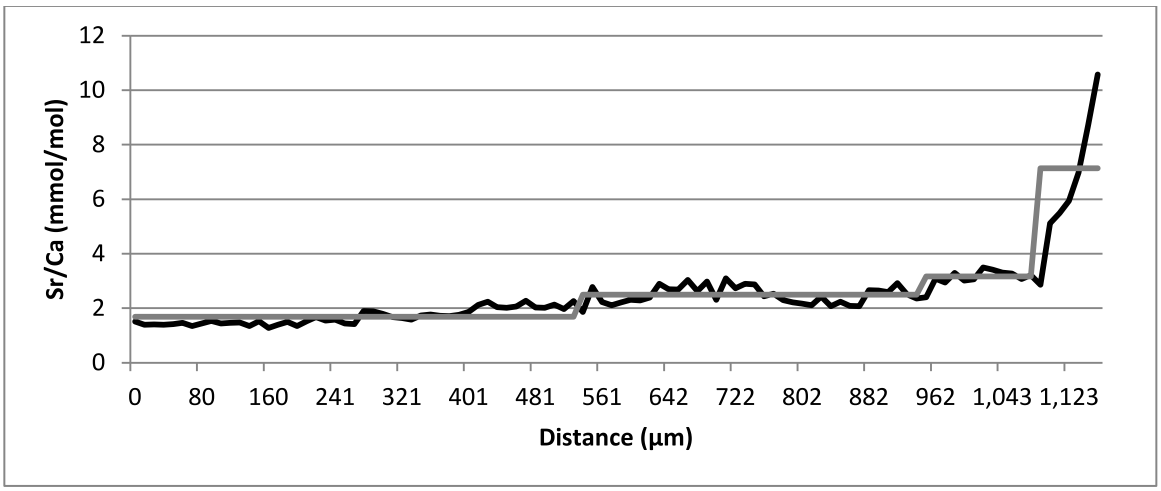
B.

C.

Figure AB.4. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 4. Figure 4.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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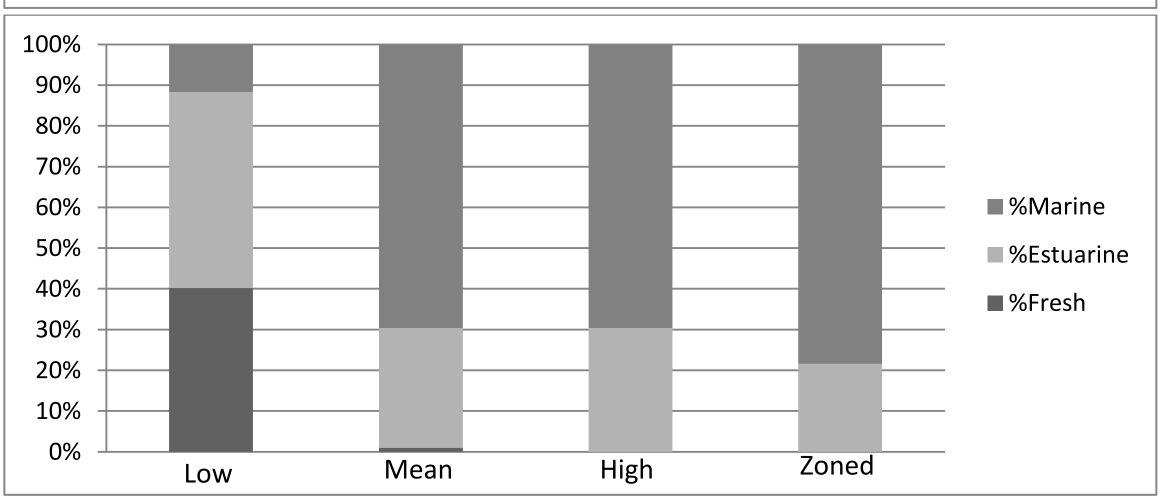


C.

Figure AB.5. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 4x. Figure 5.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

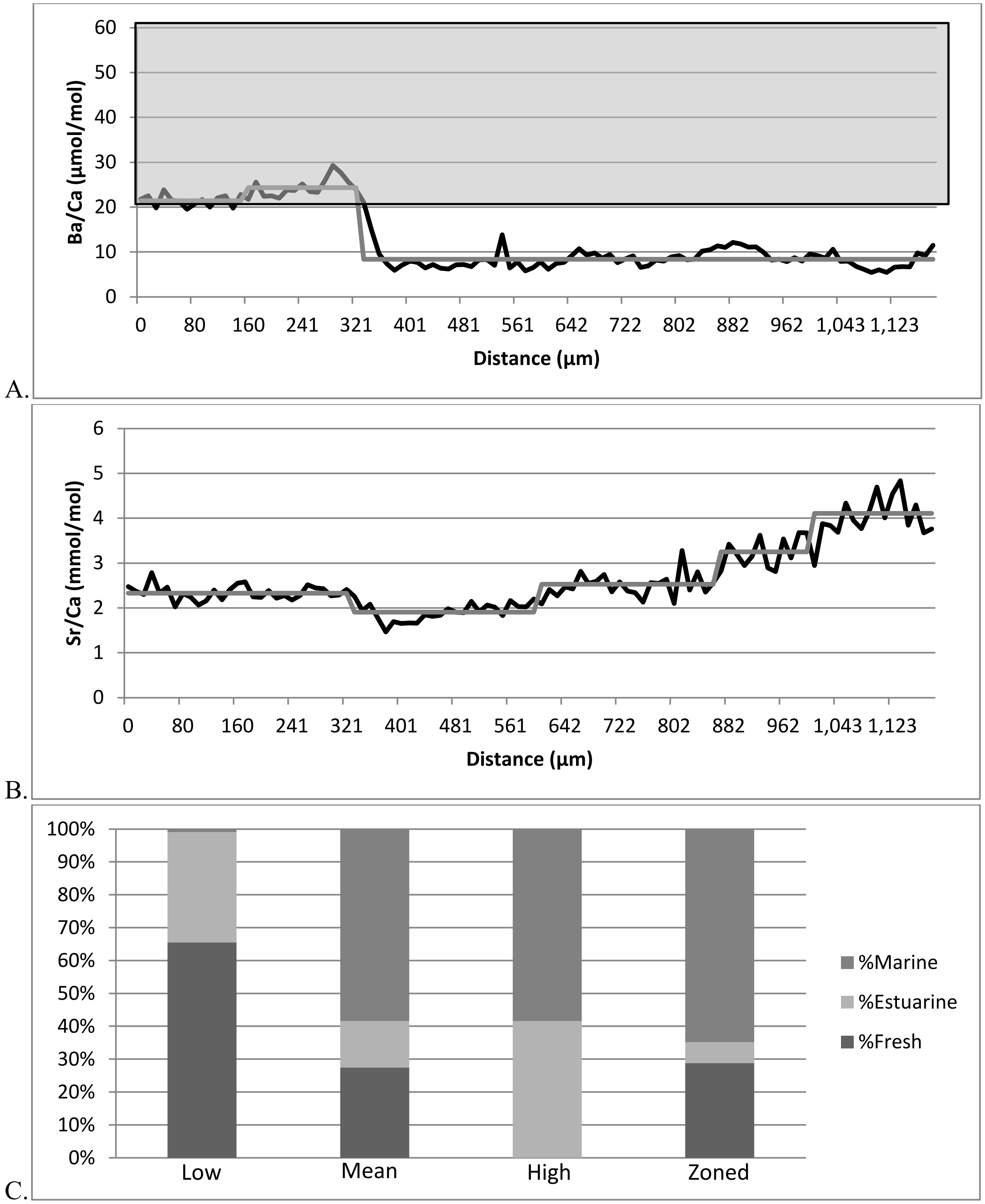
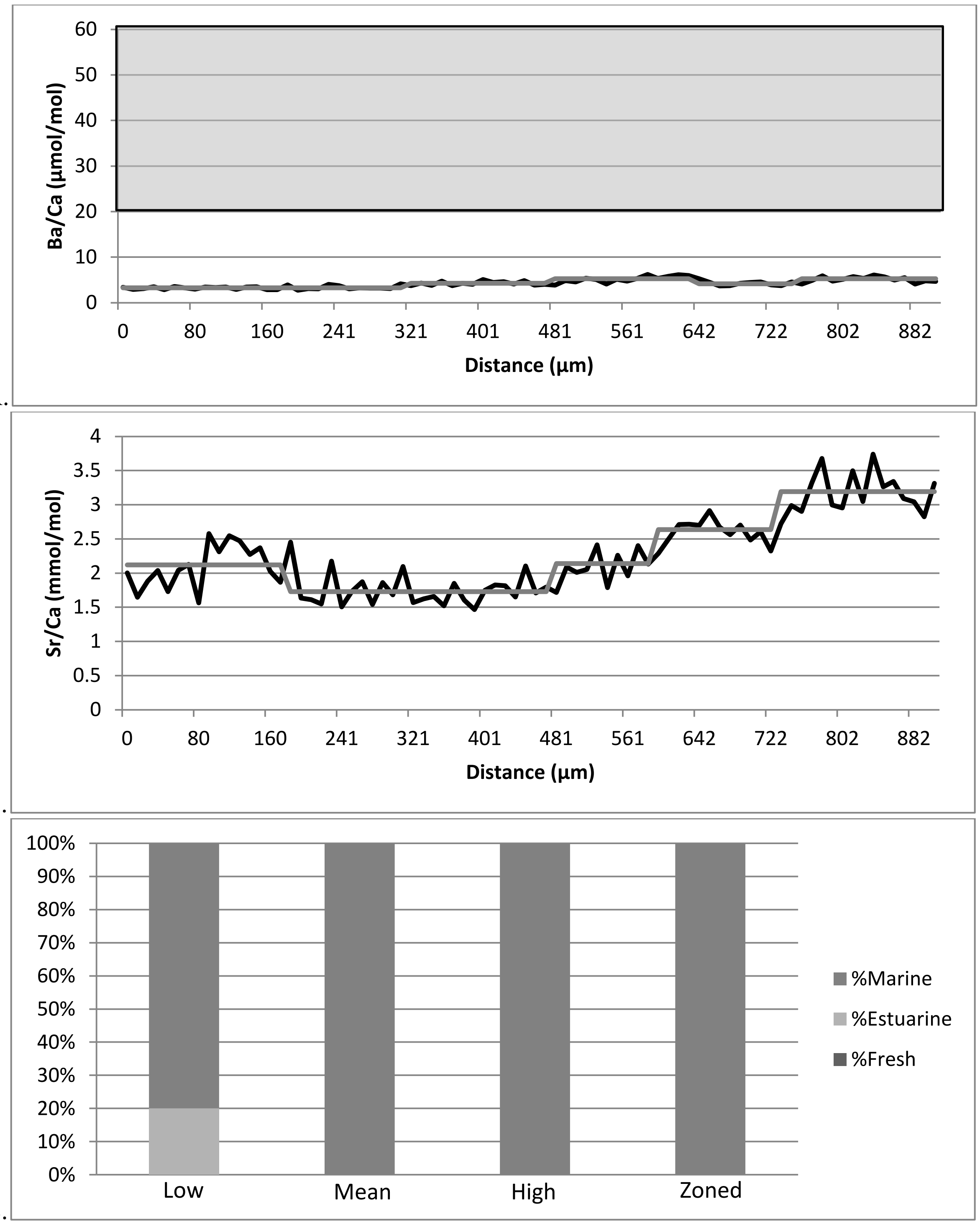
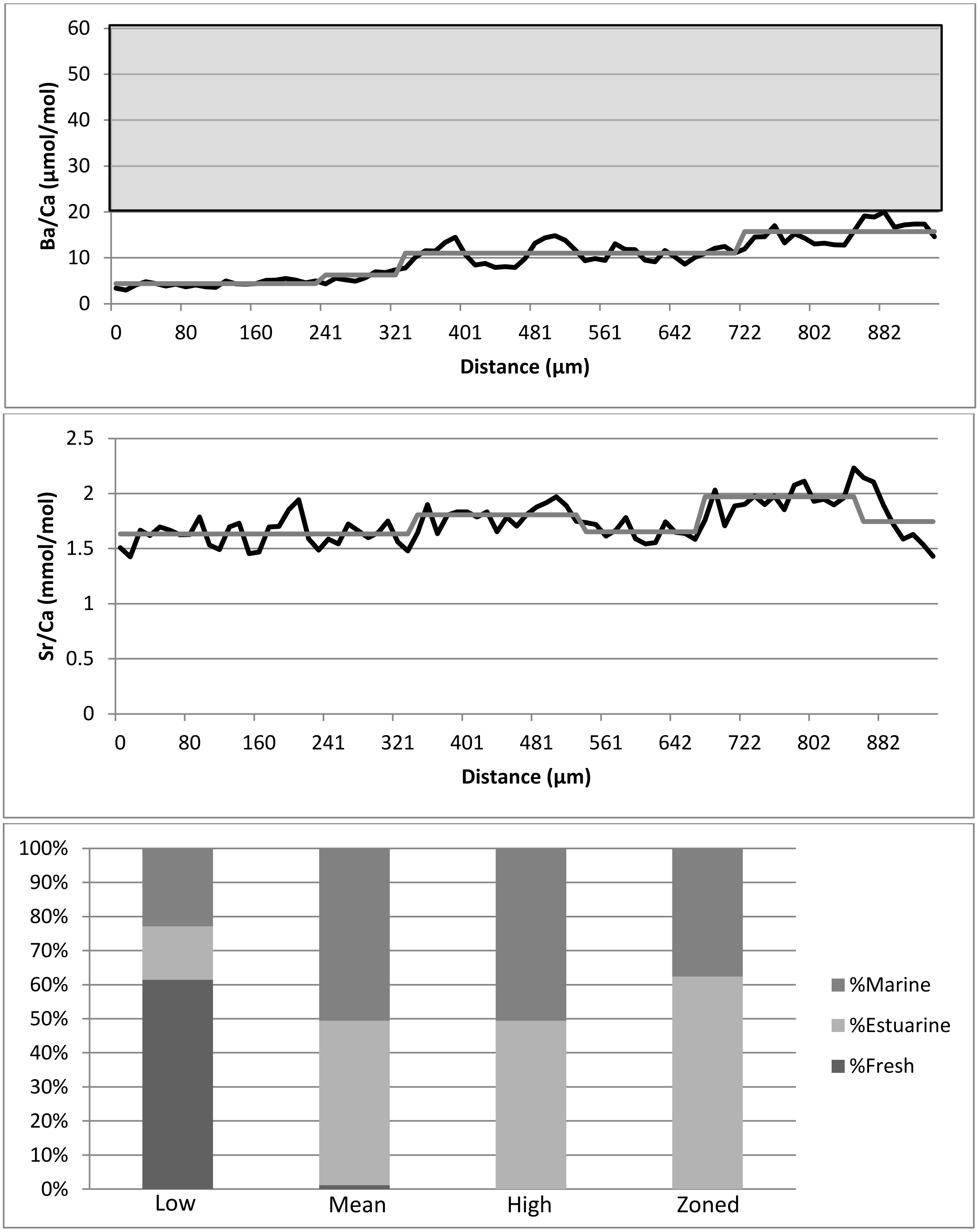


Figure AB.6. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 6. Figure 6.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



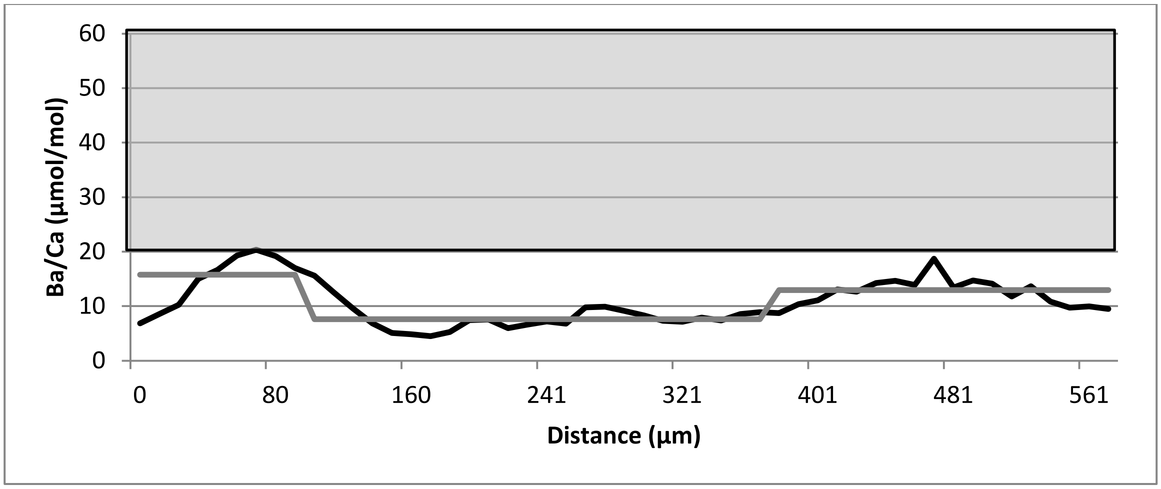
A.
B.
C.

Figure AB.7. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 7. Figure 7.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

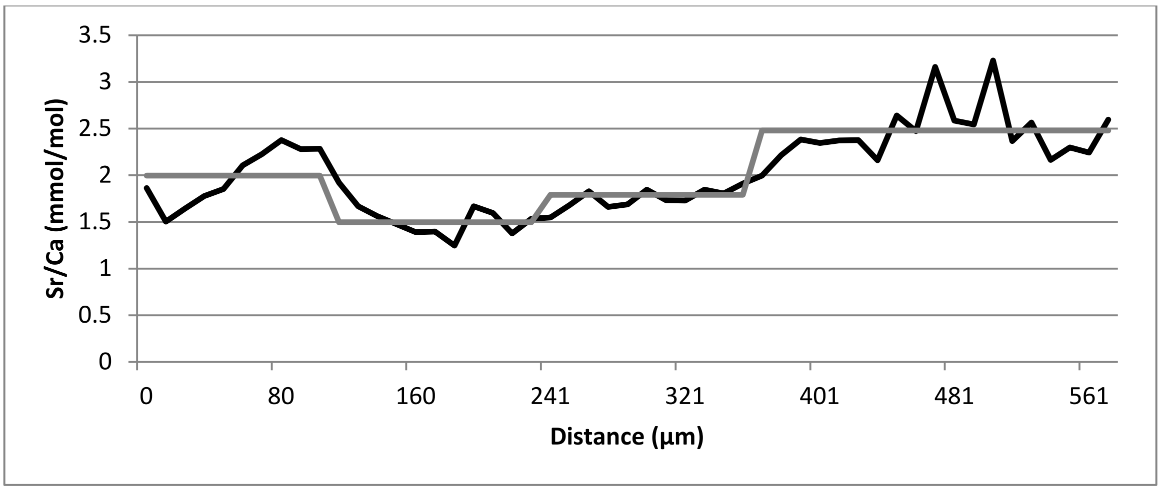


A.
B.
C.

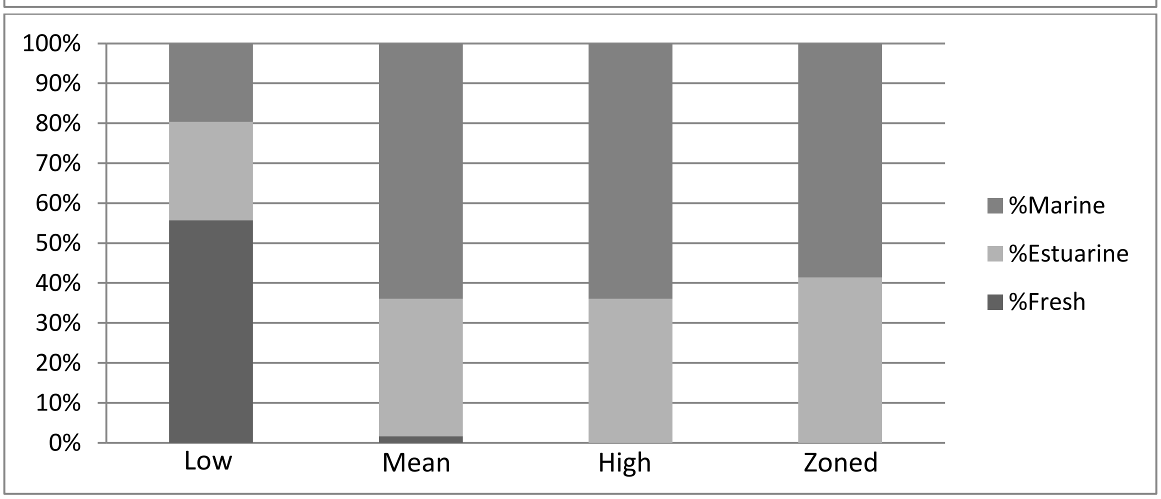
Figure AB.8. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 8. Figure 8.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.9. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 9. Figure 9.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

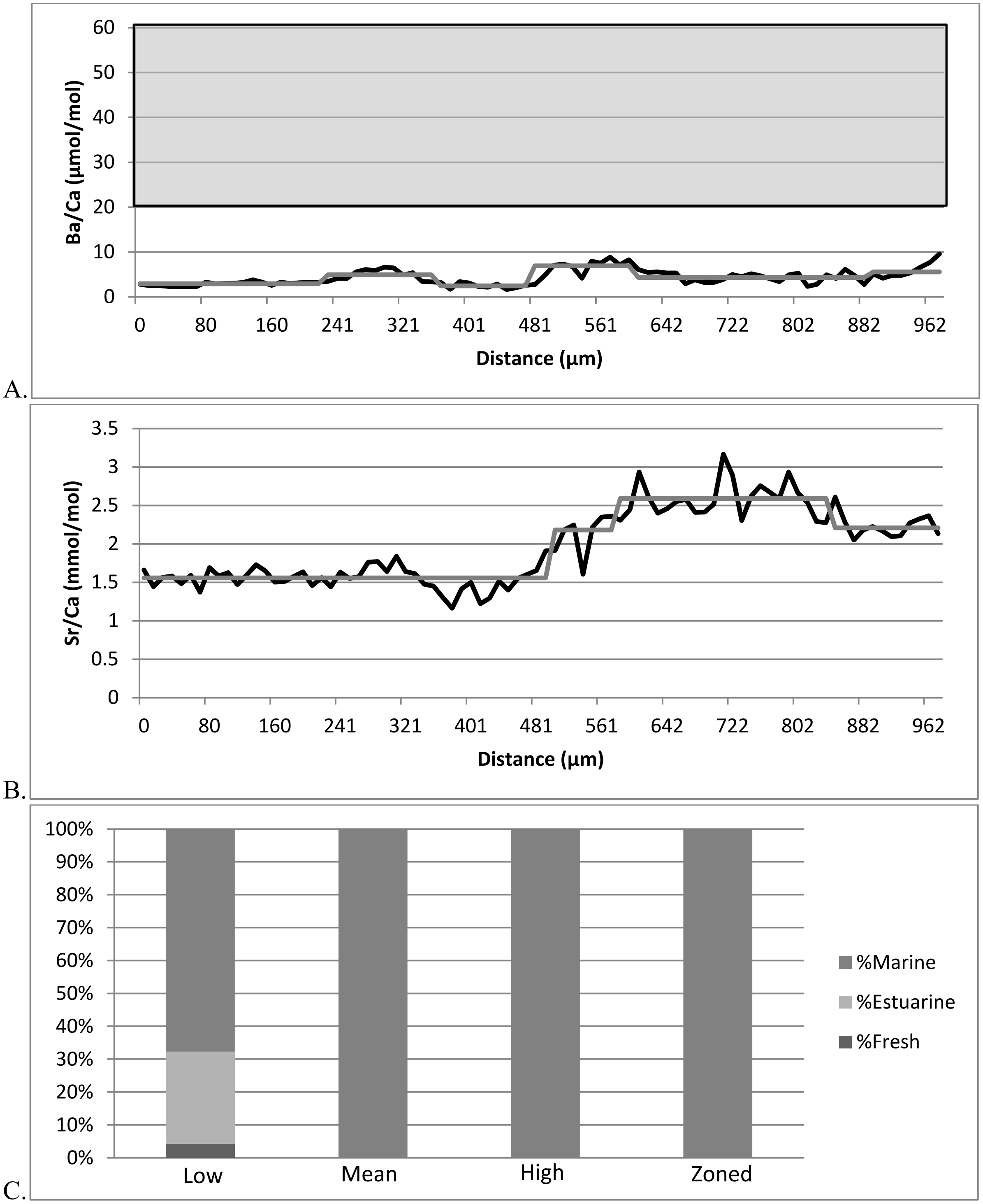
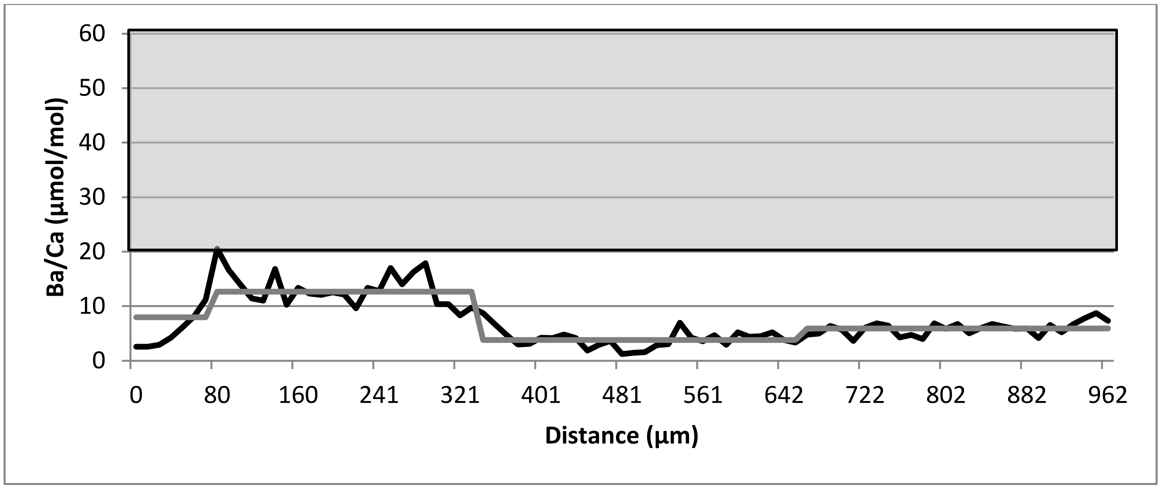
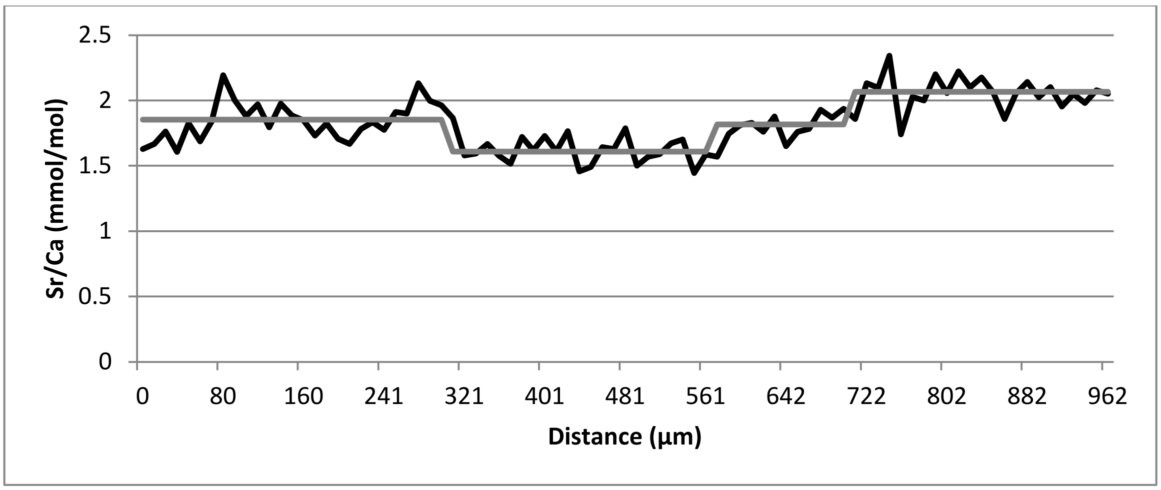


Figure AB.10. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 10. Figure 10.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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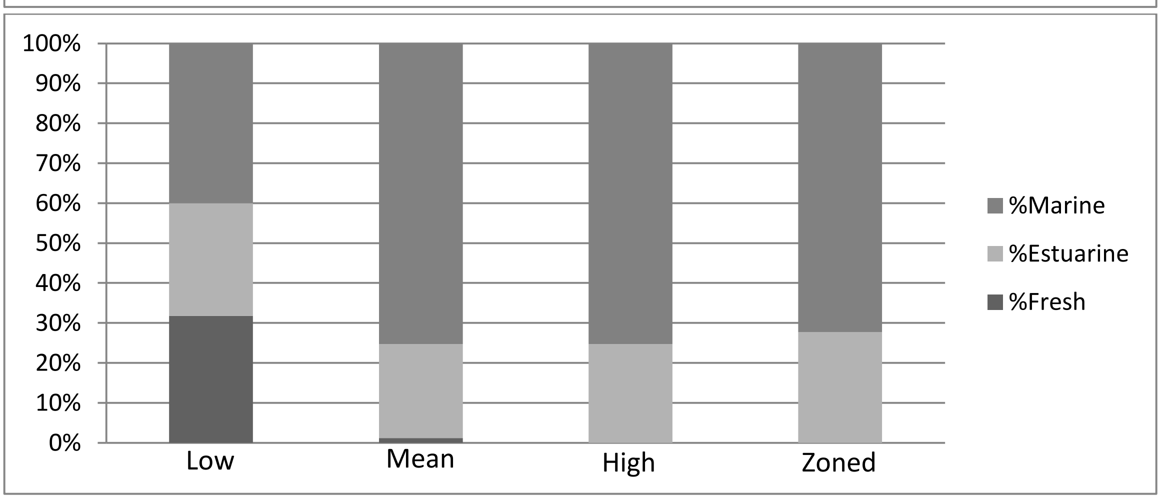
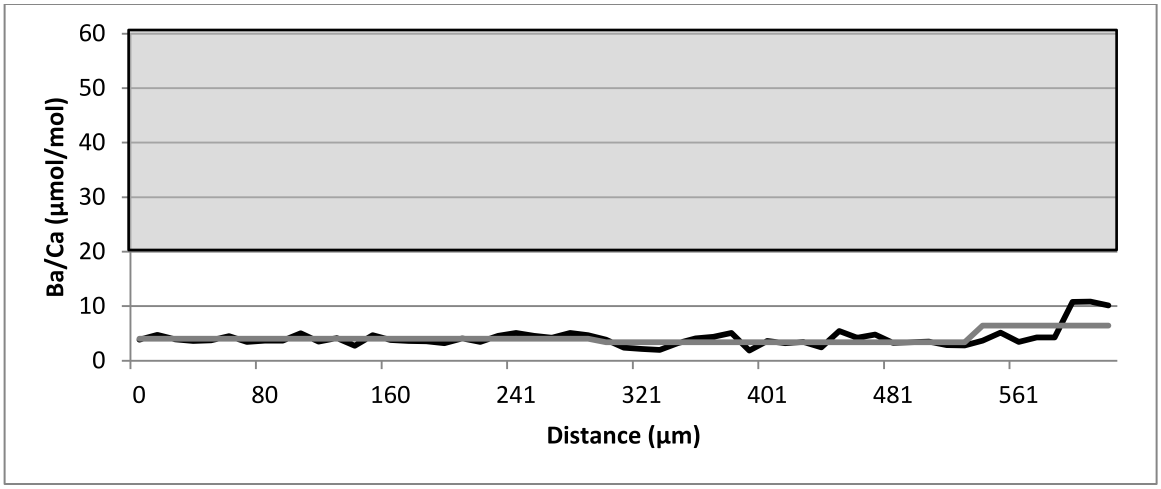
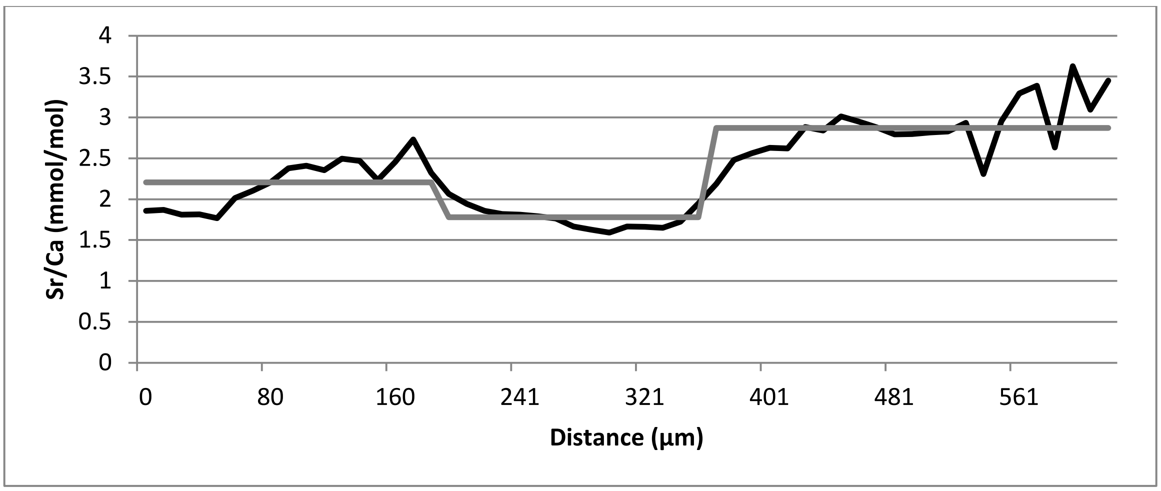


C.

Figure AB.11. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 12. Figure 11.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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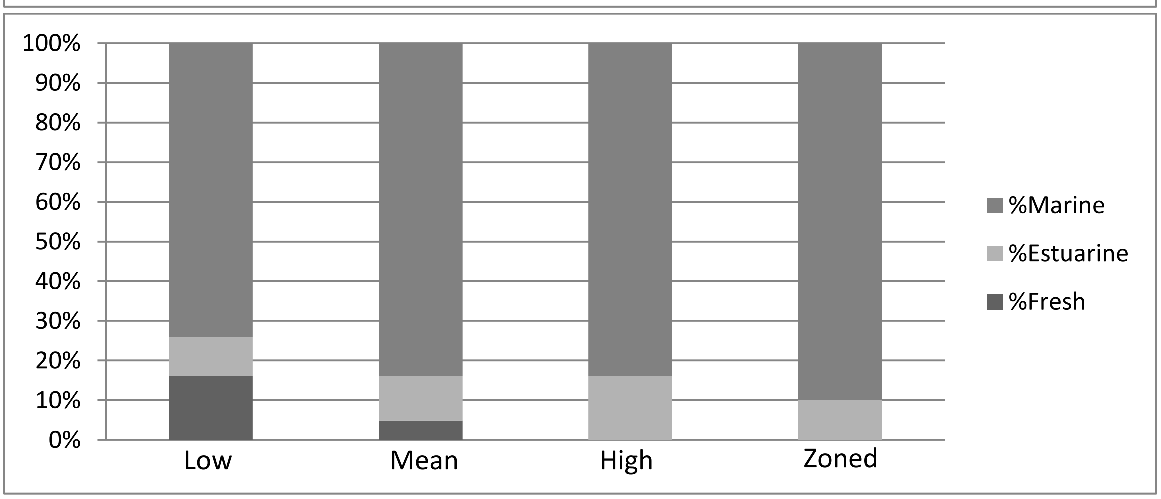
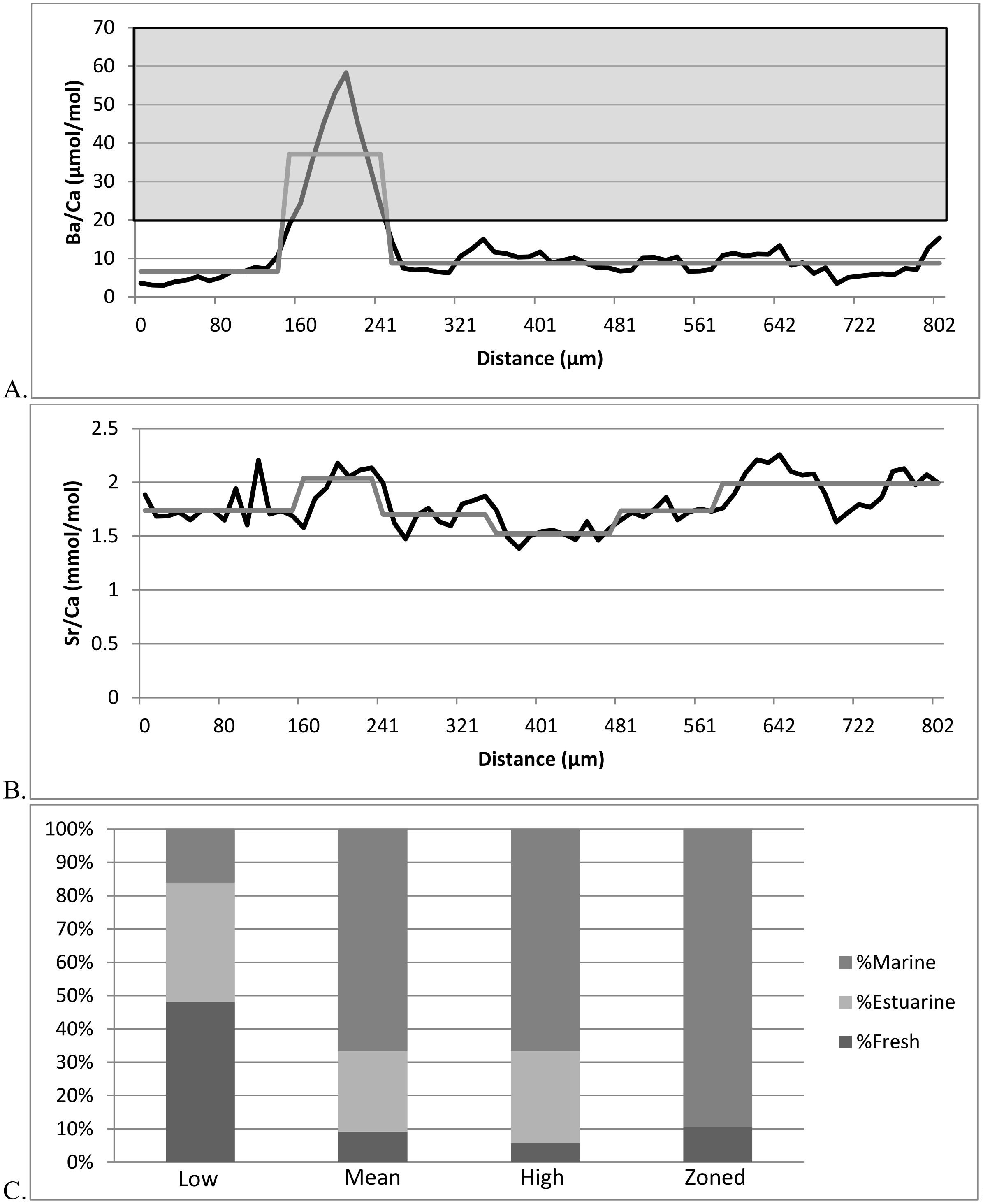


C.

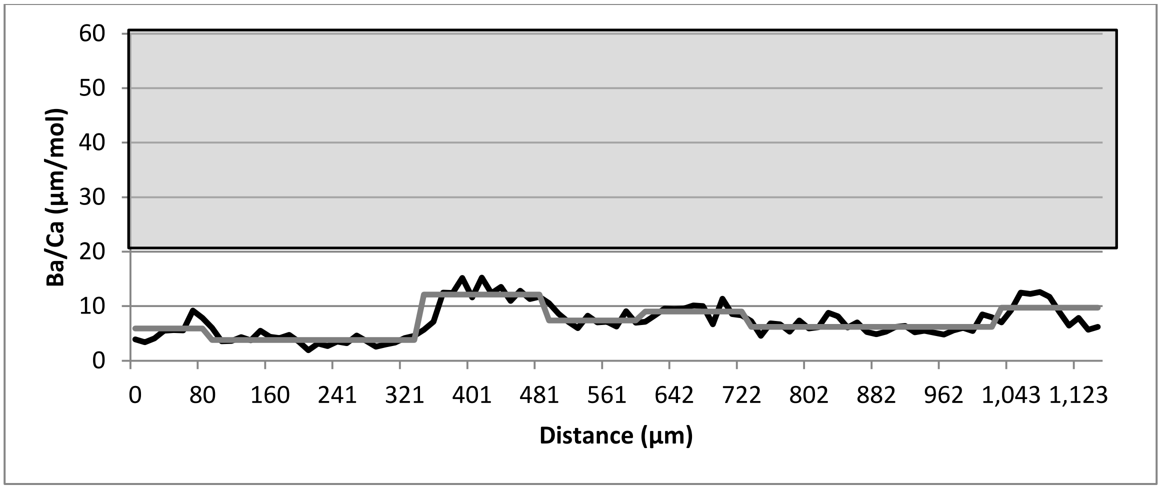
Figure AB.12. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 13. Figure 12.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



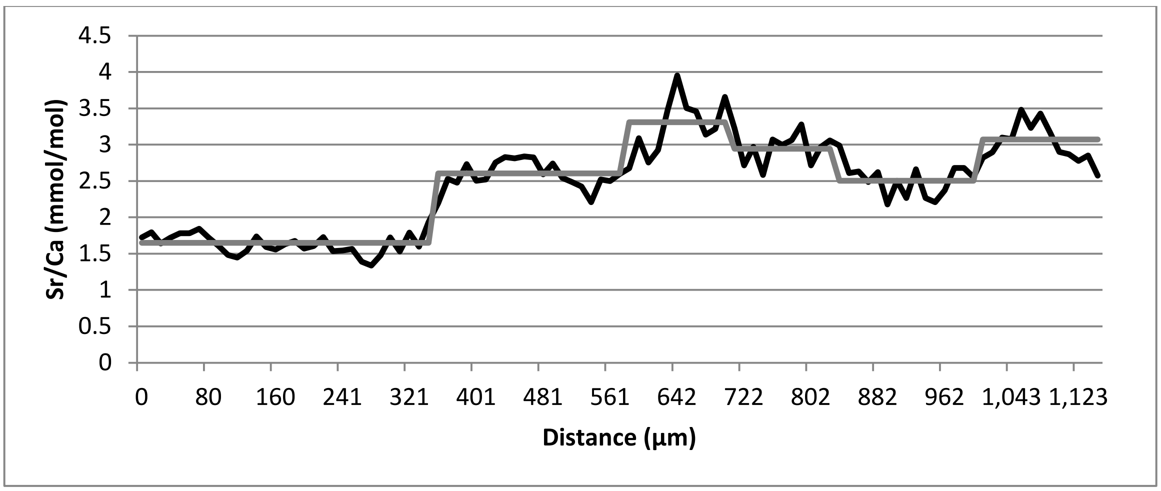
s

Figure AB.13. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 15. Figure 13.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.



B.



C.

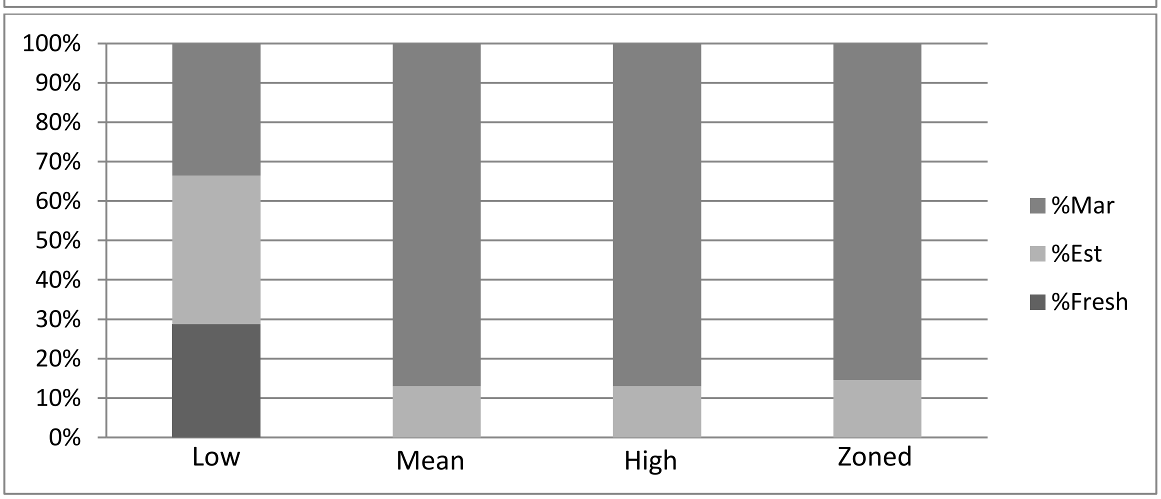
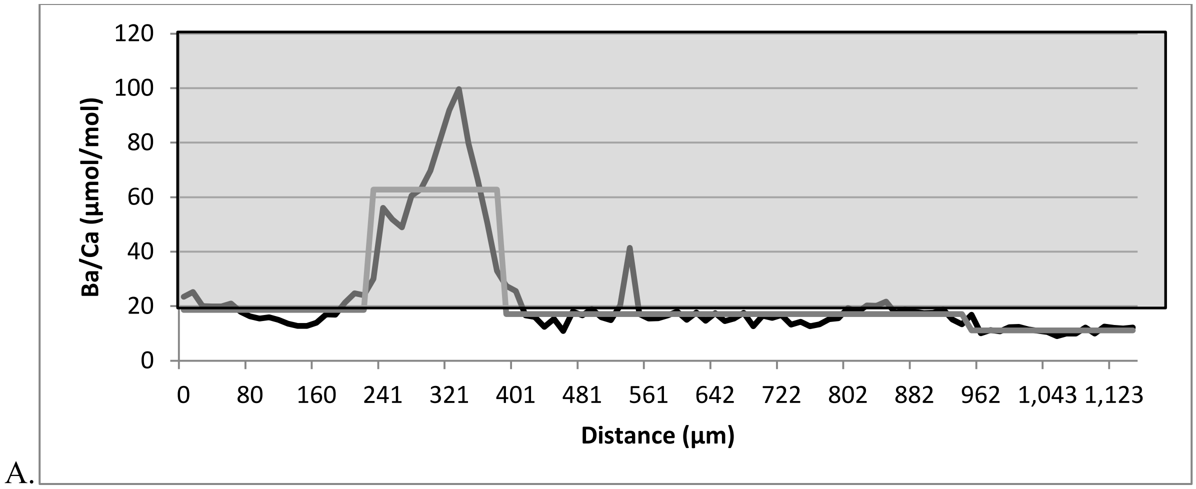
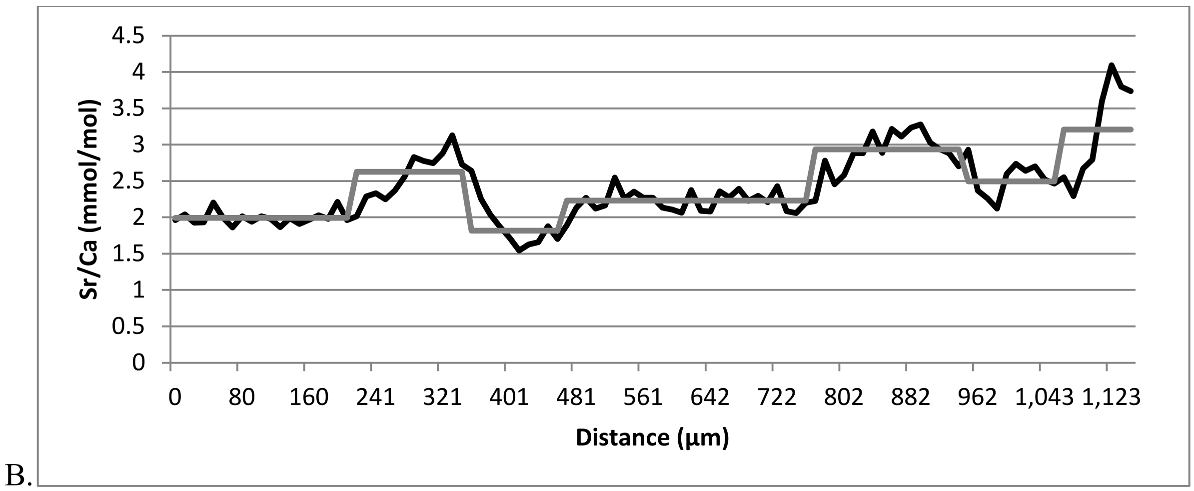


Figure AB.14. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 16. Figure 14.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.





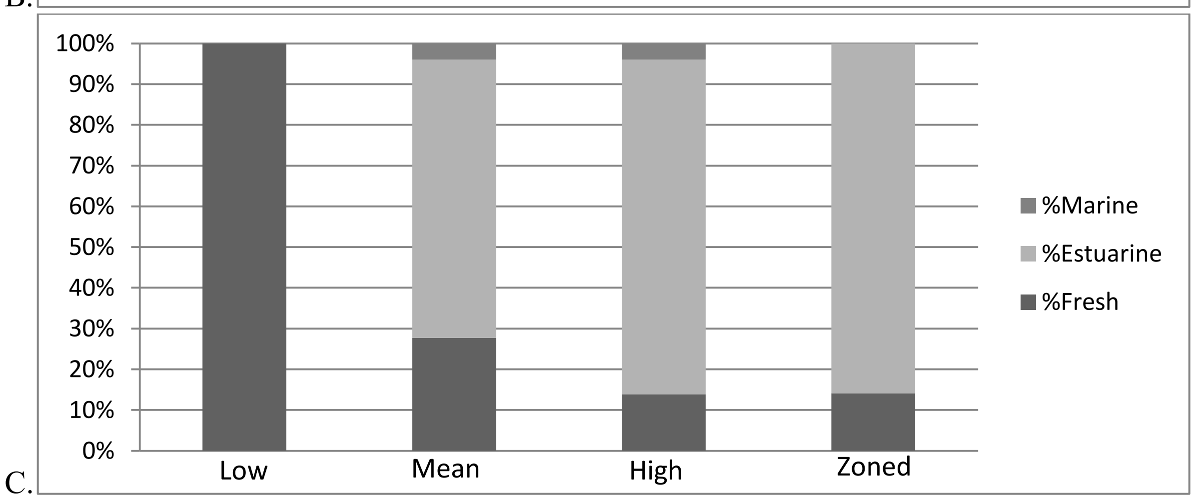
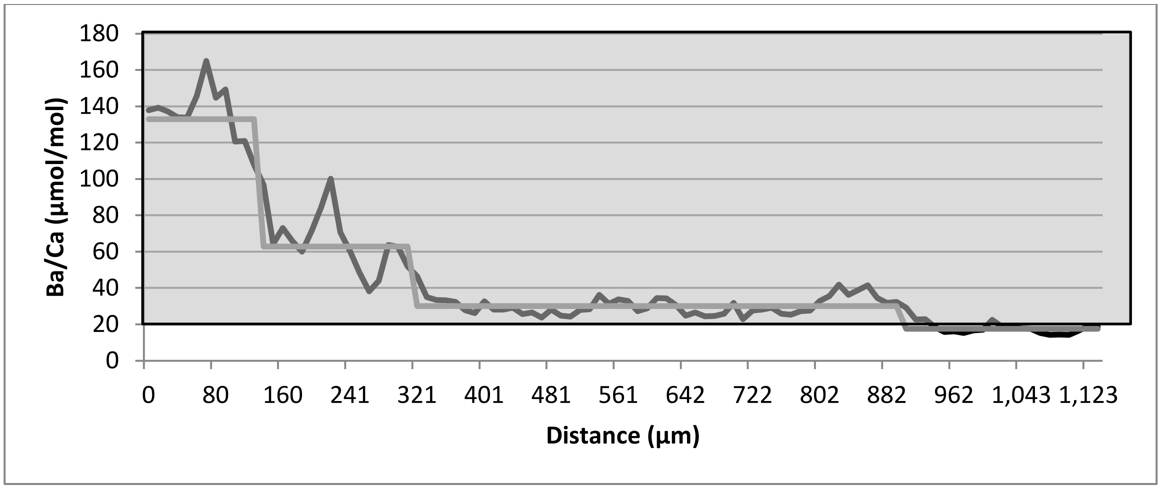
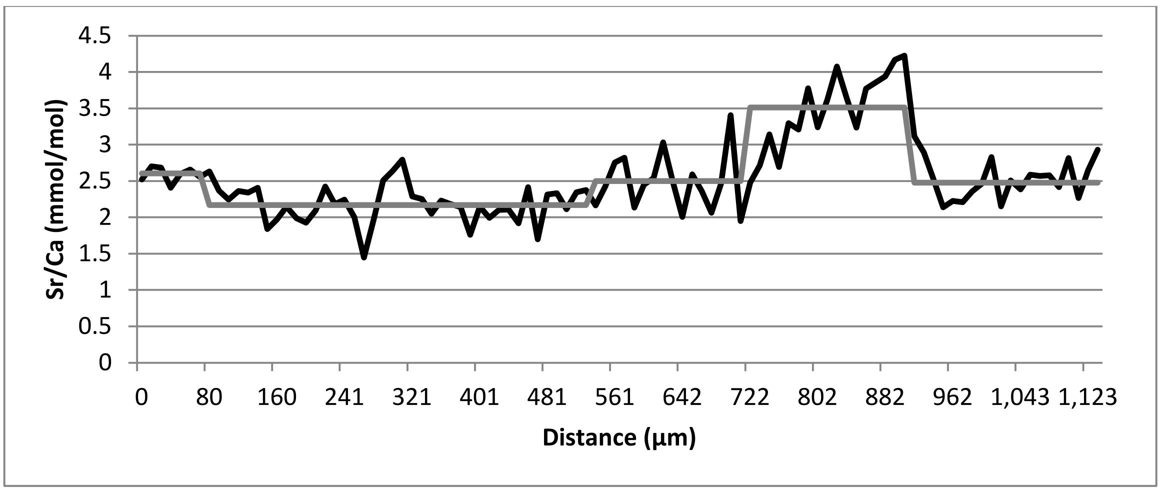


Figure AB.15. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 17. Figure 15.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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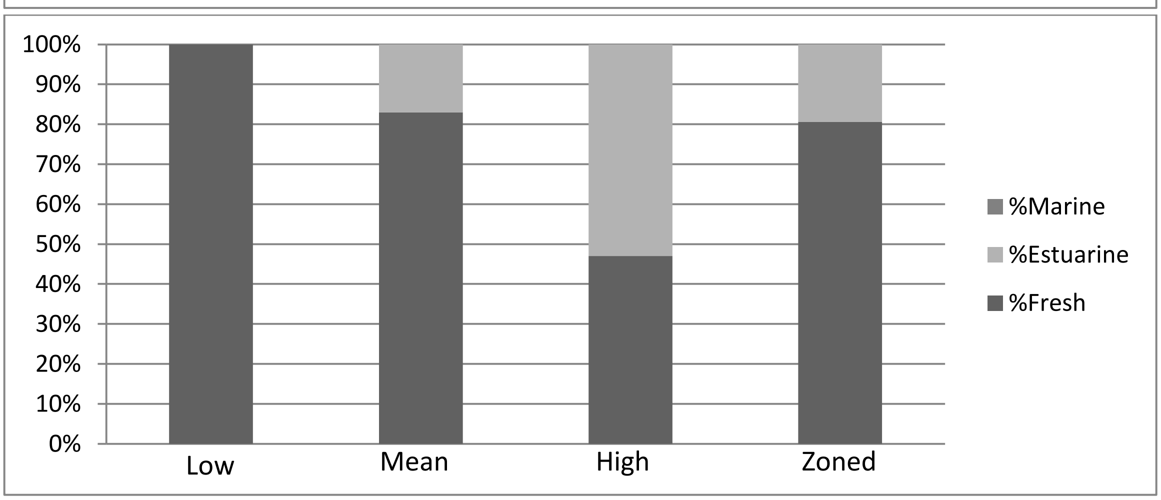
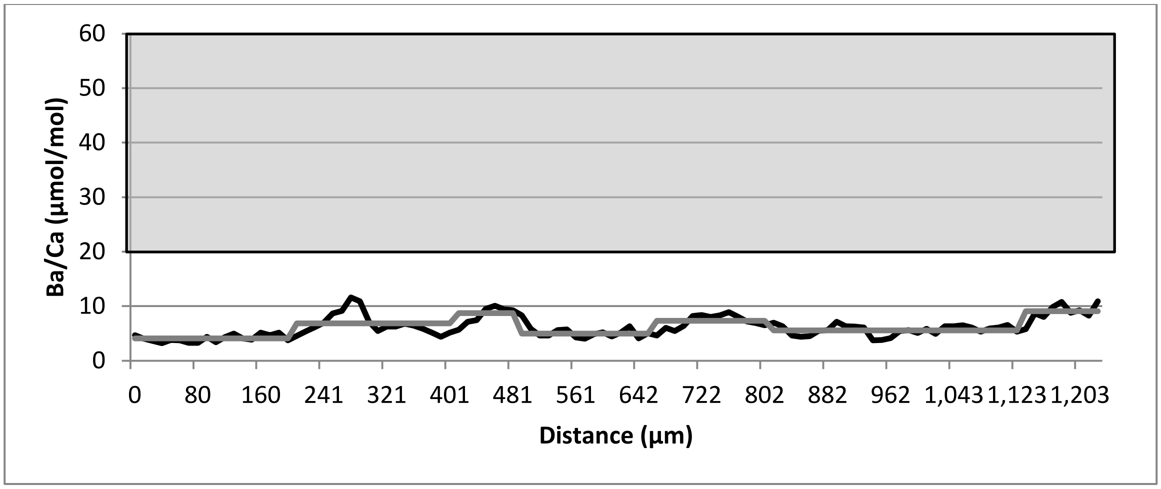
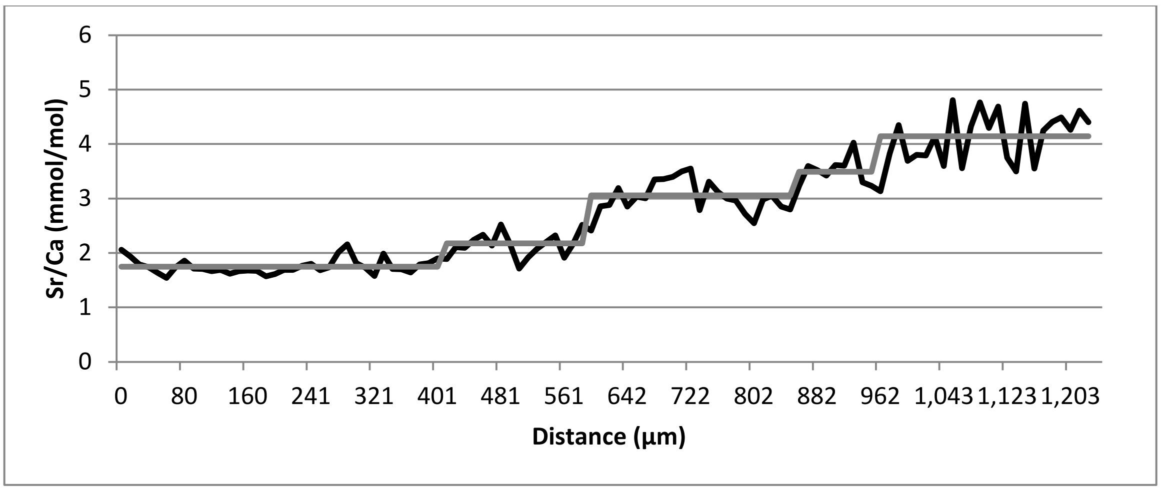


C.

Figure AB.16. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 19. Figure 16.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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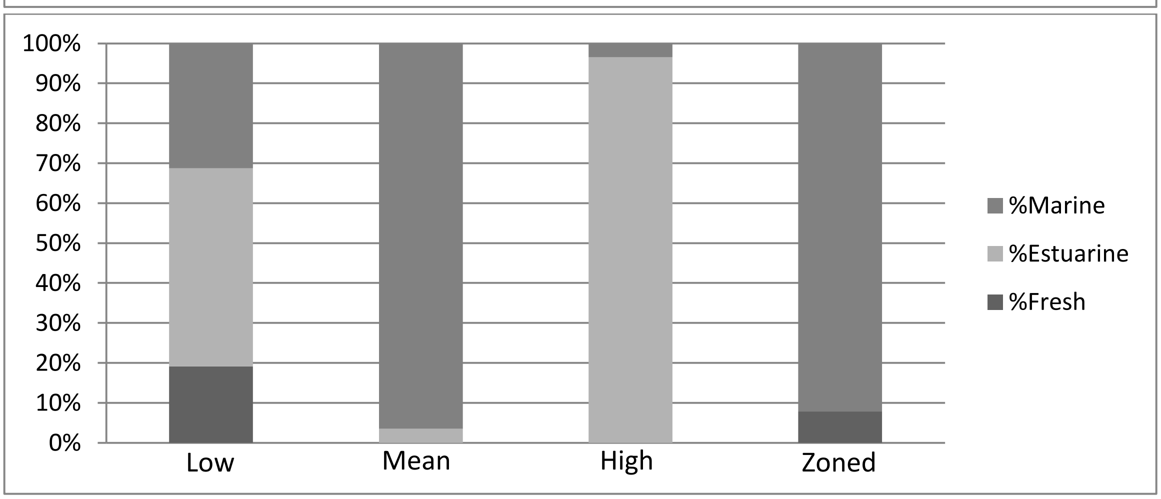


C.

Figure AB.17. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 20. Figure 17.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

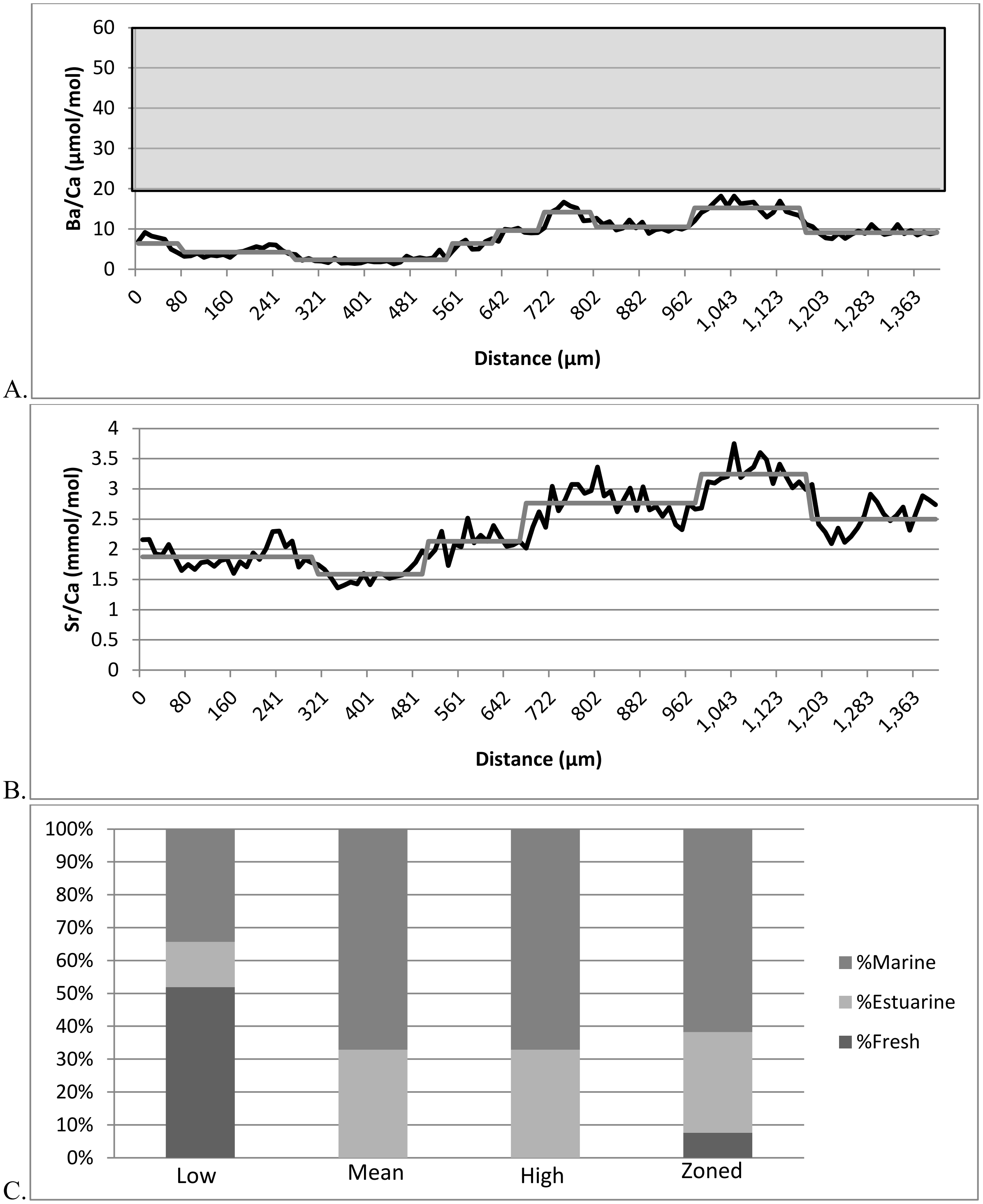
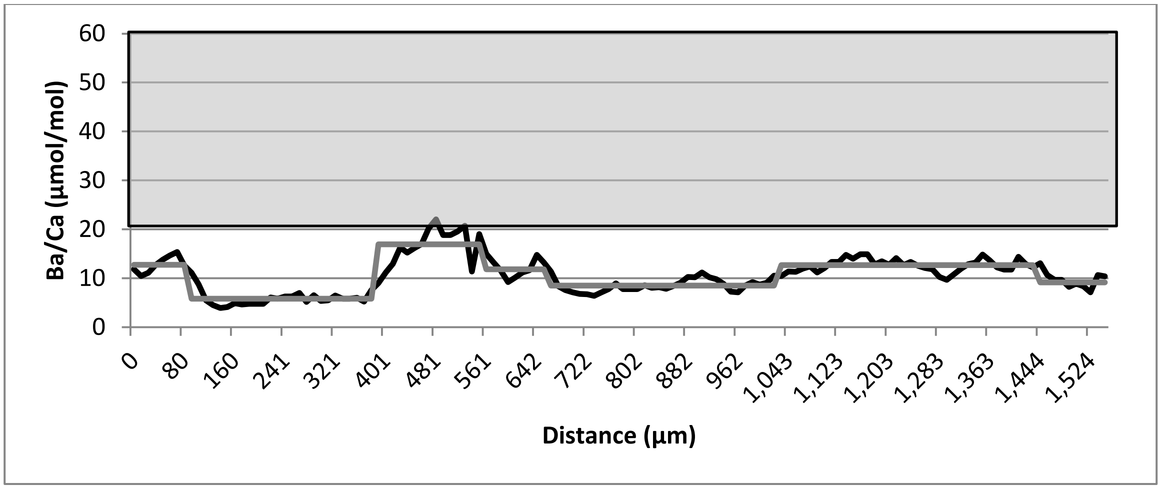
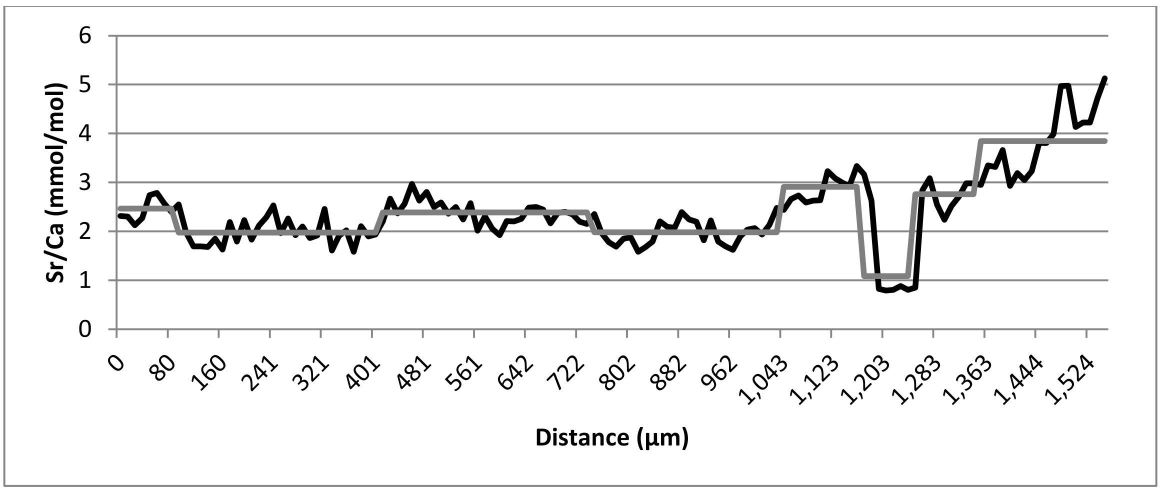


Figure AB.18. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 21. Figure 18.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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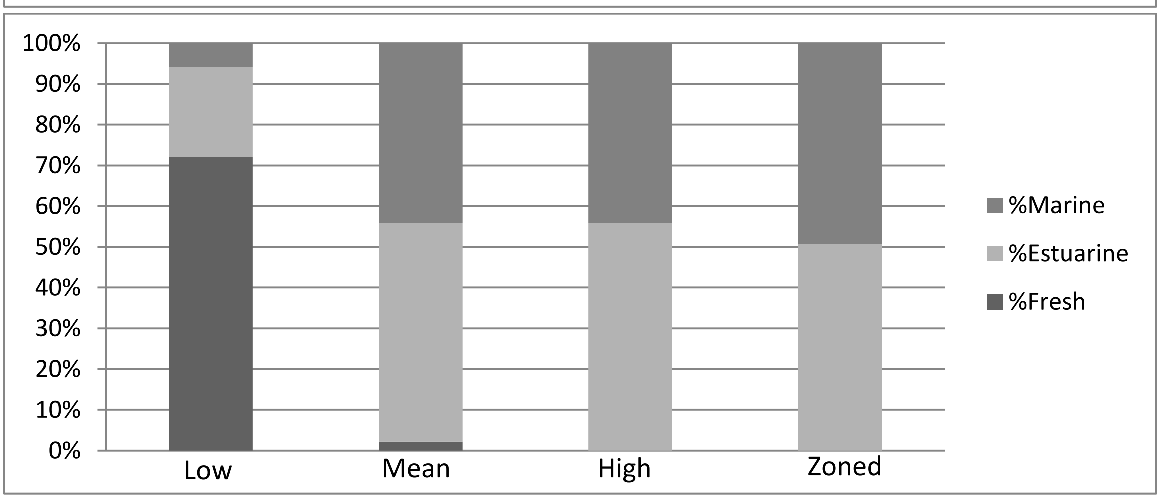
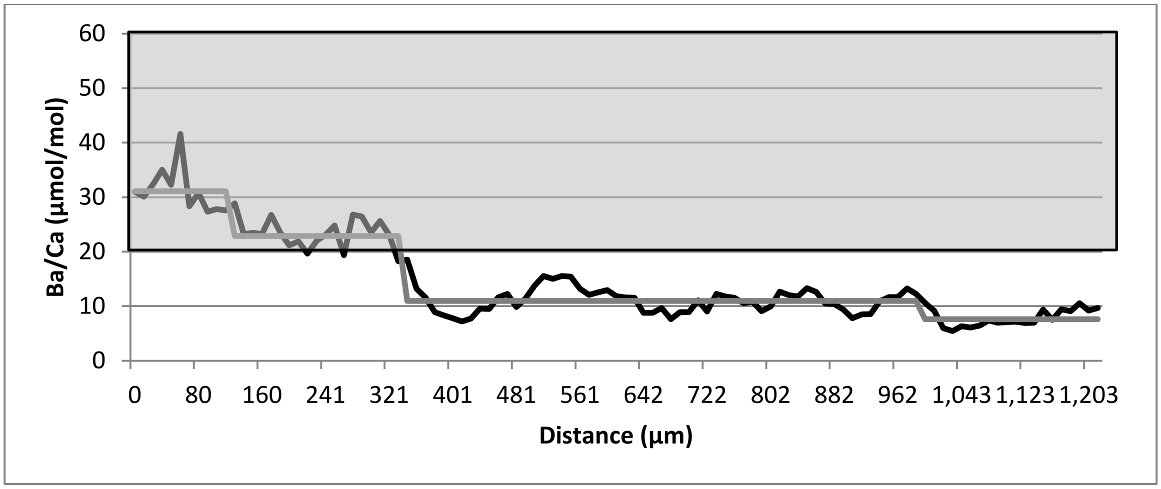


C.

Figure AB.19. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 22. Figure 19.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


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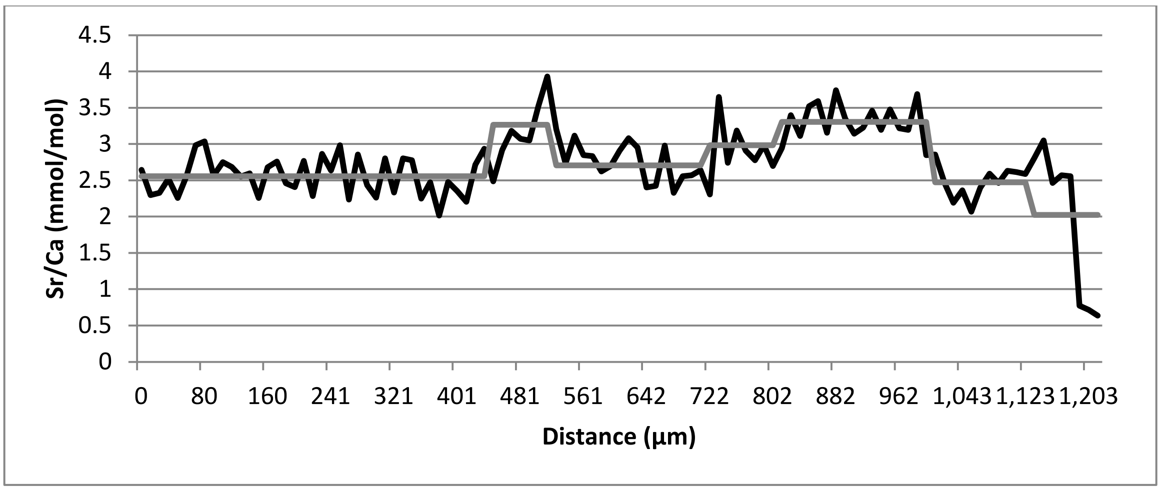


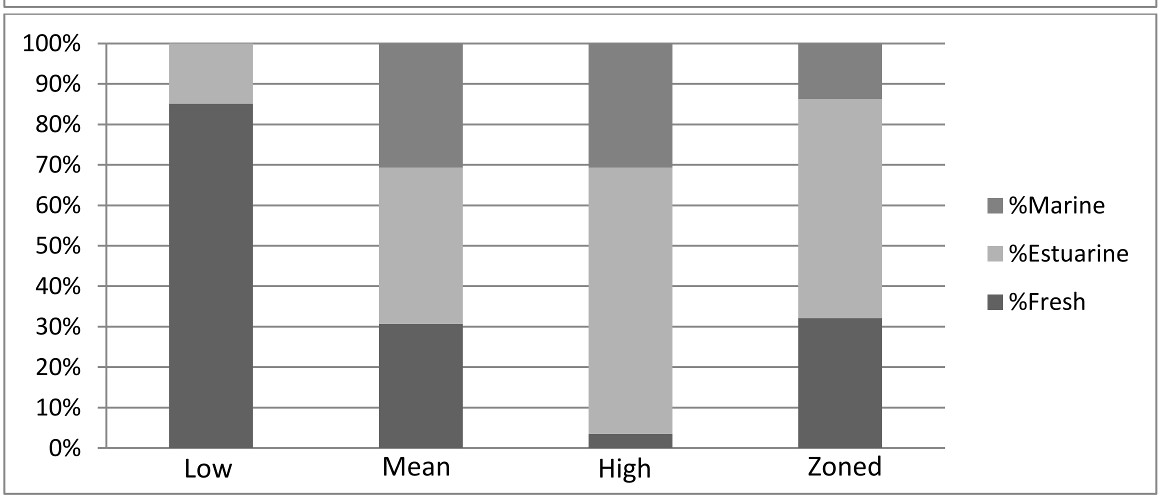
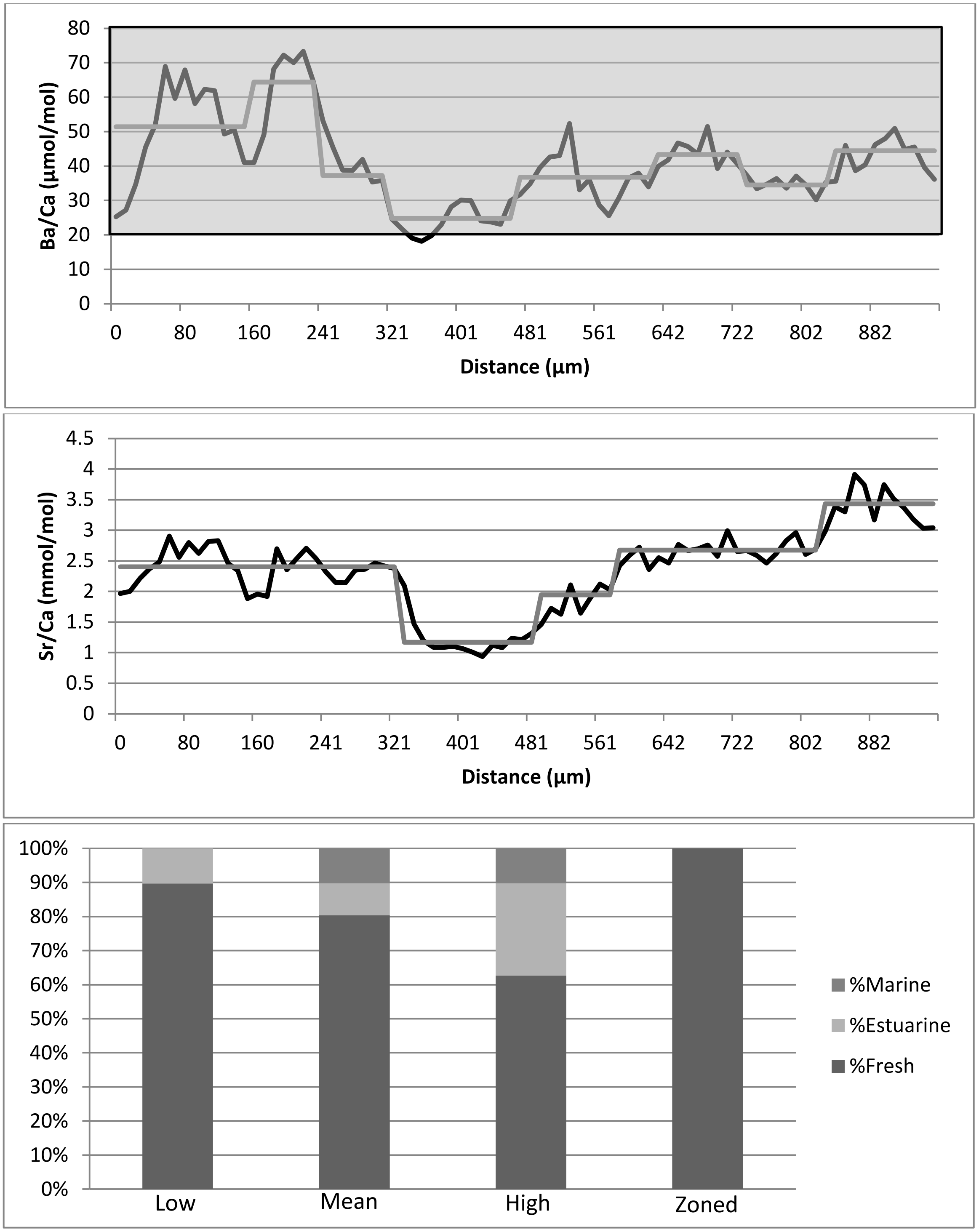
C.


Figure AB.20. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 23. Figure 20.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

B.

C.

Figure AB.21. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 32. Figure 21.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

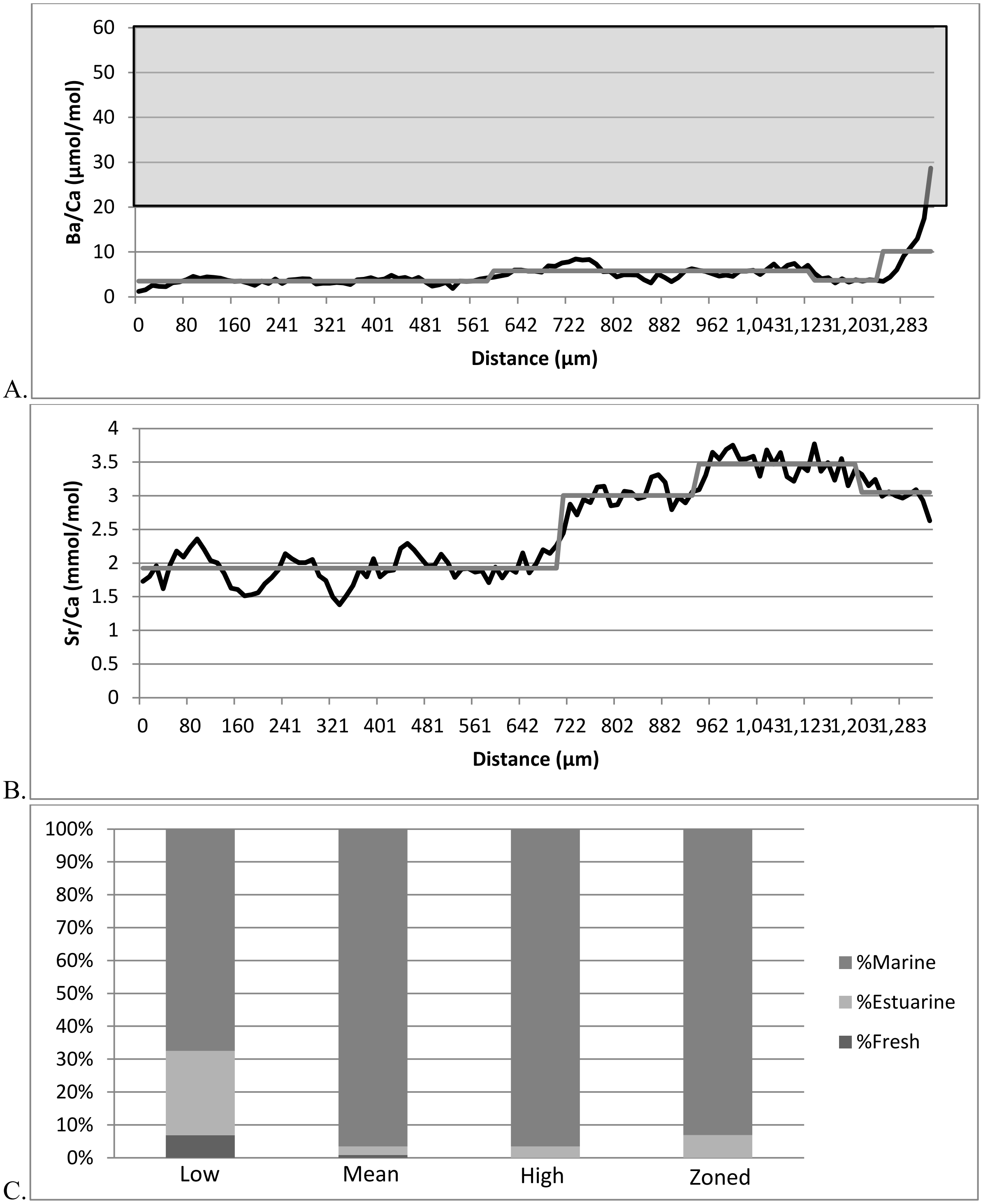
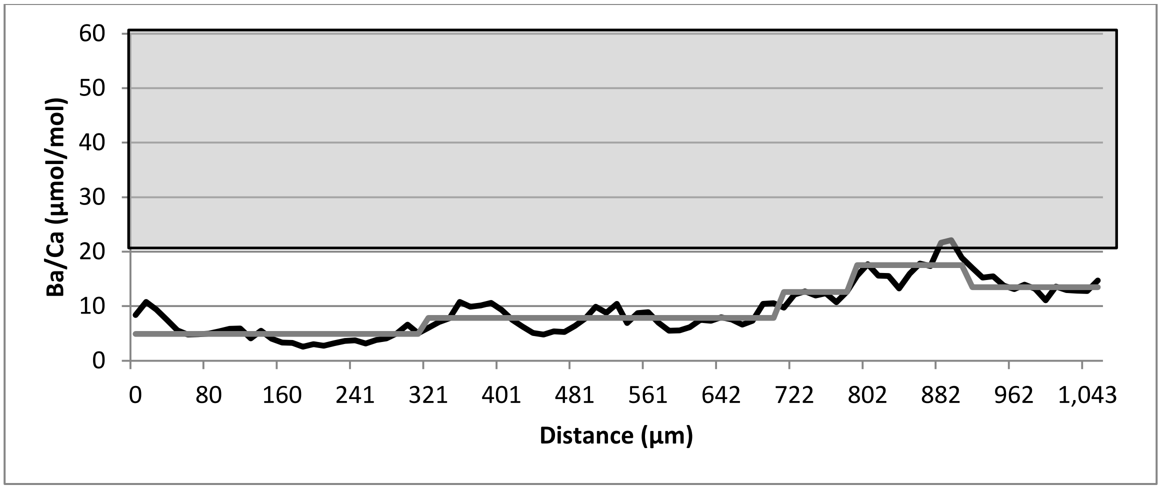
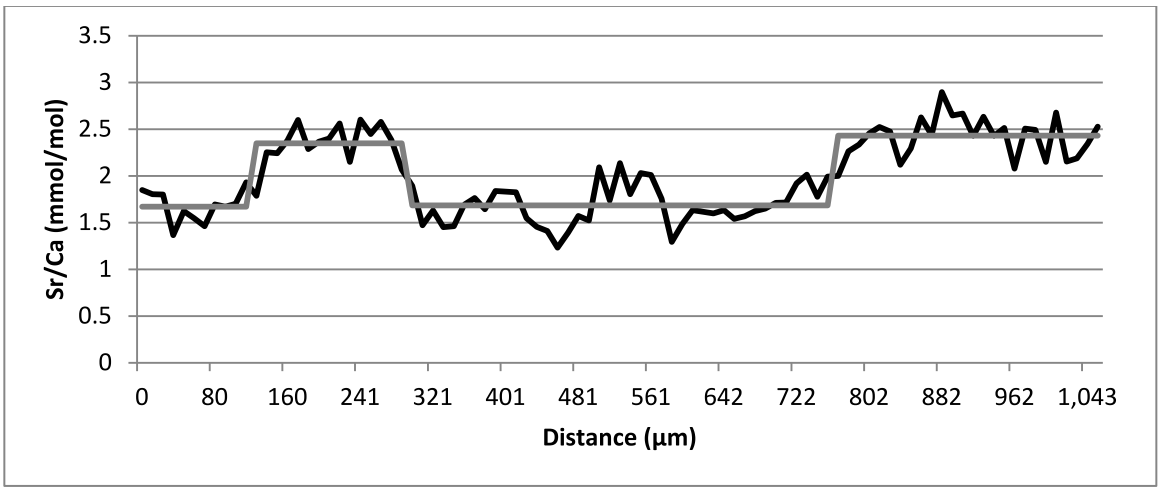


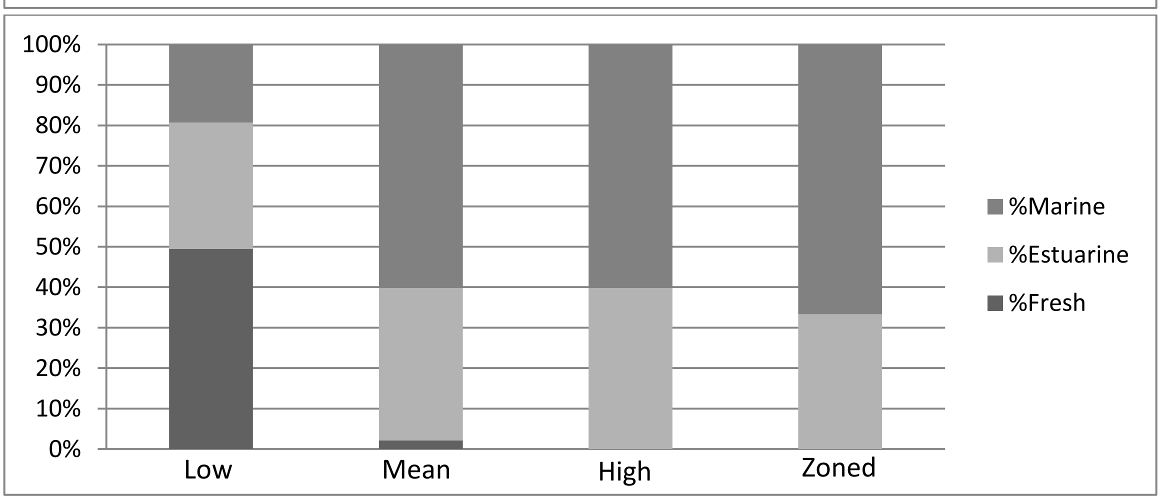
Figure AB.22. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 35. Figure 22.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.23. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 36. Figure 23.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

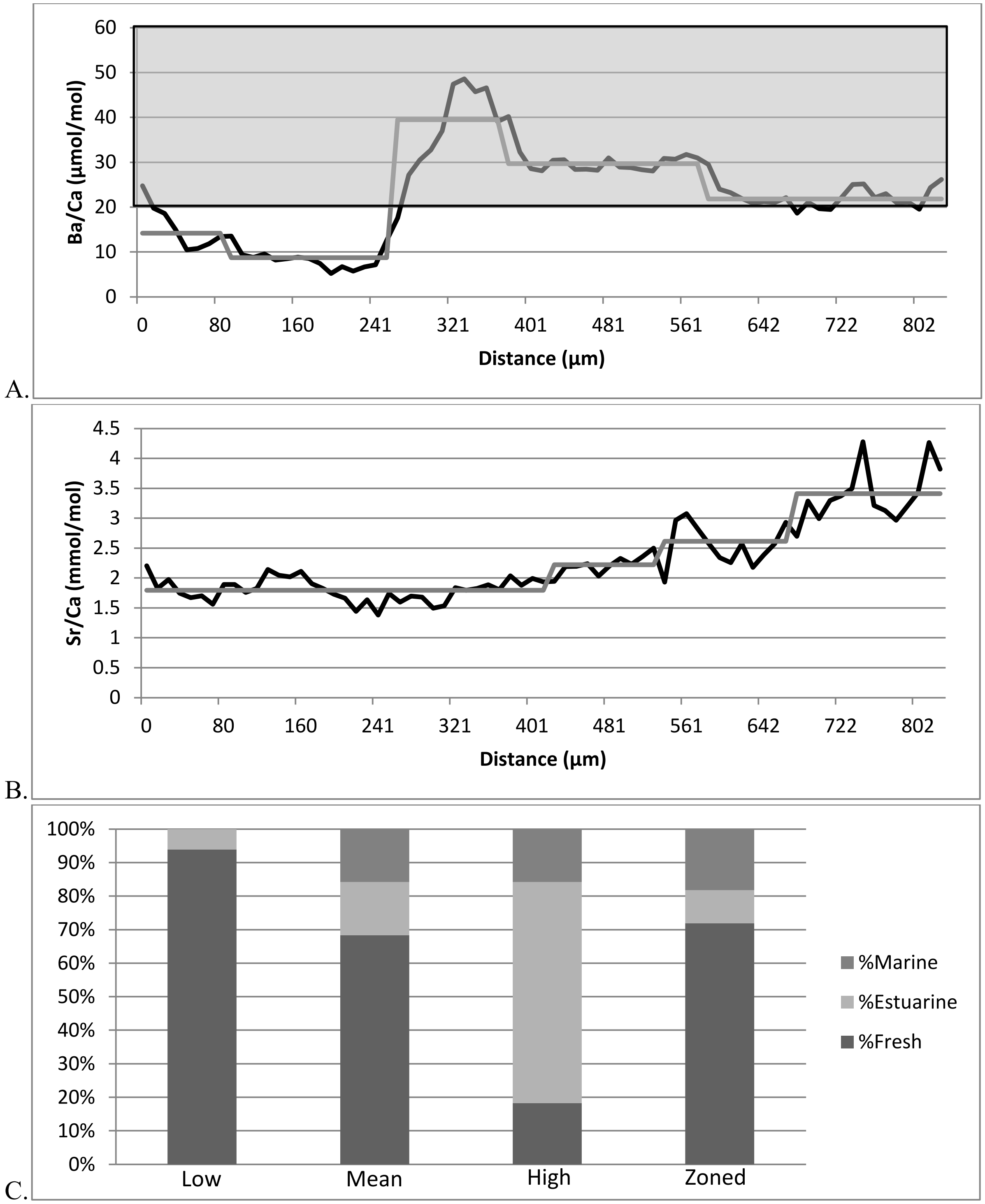
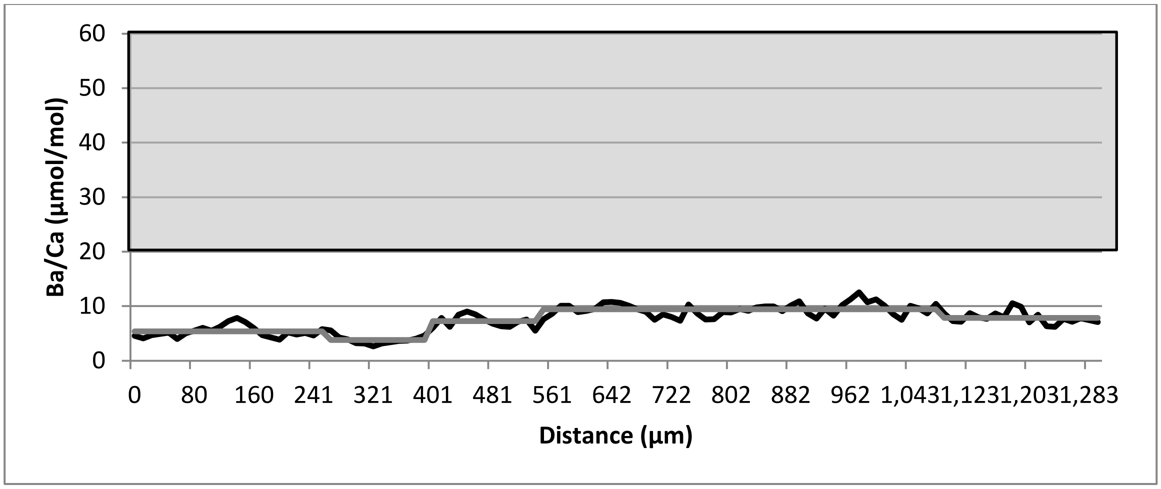
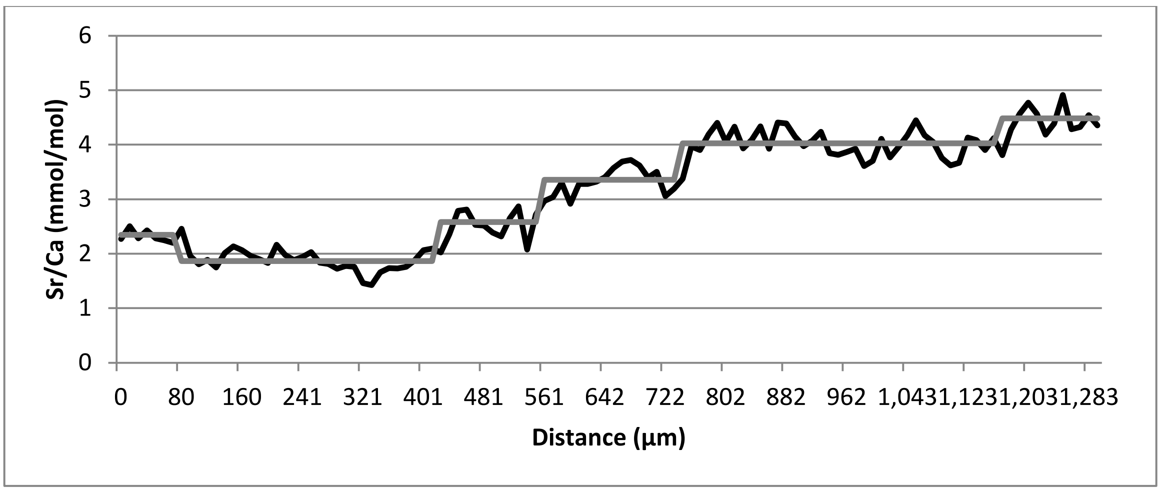


Figure AB.24. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 37. Figure 24.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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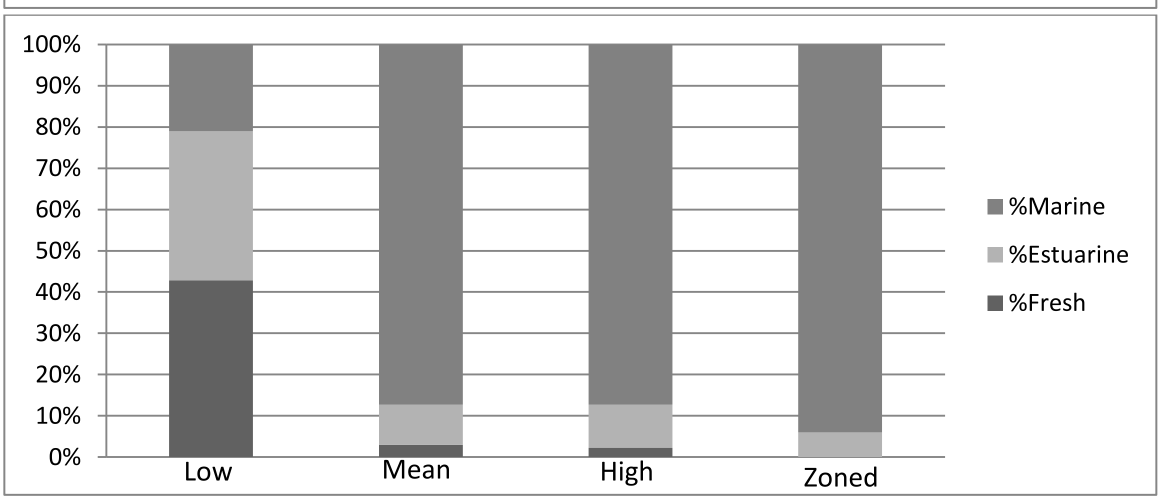
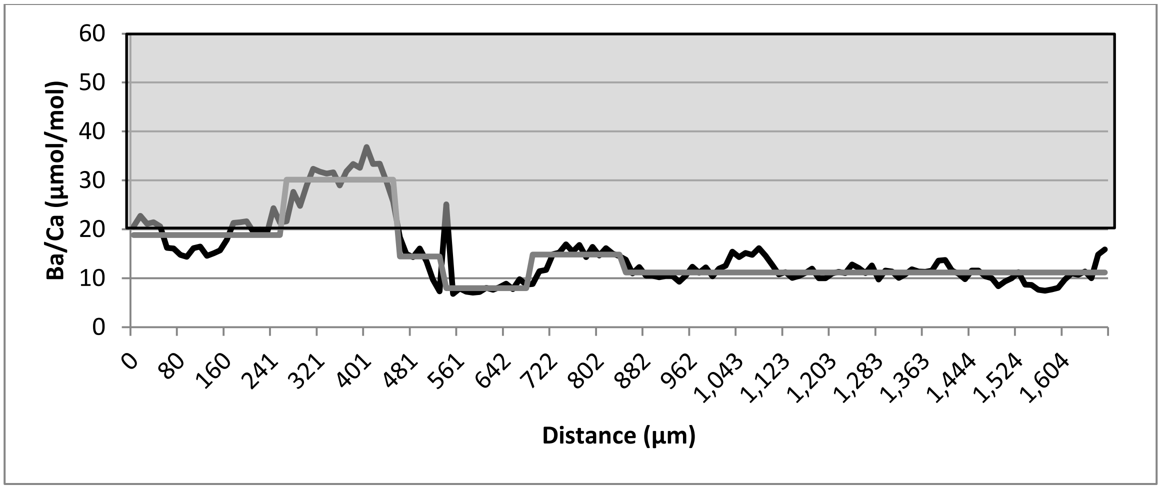
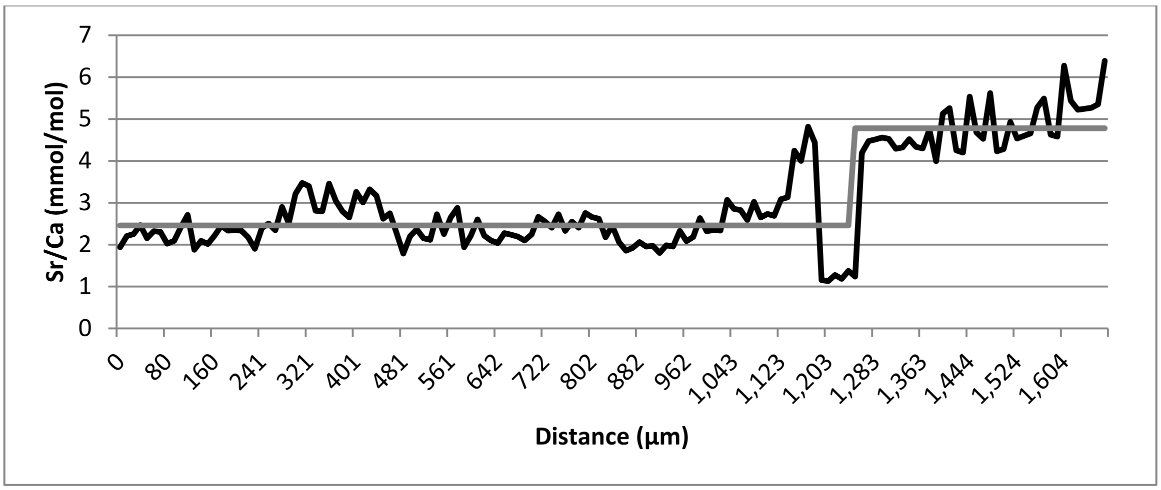


C.

Figure AB.25. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 38. Figure 25.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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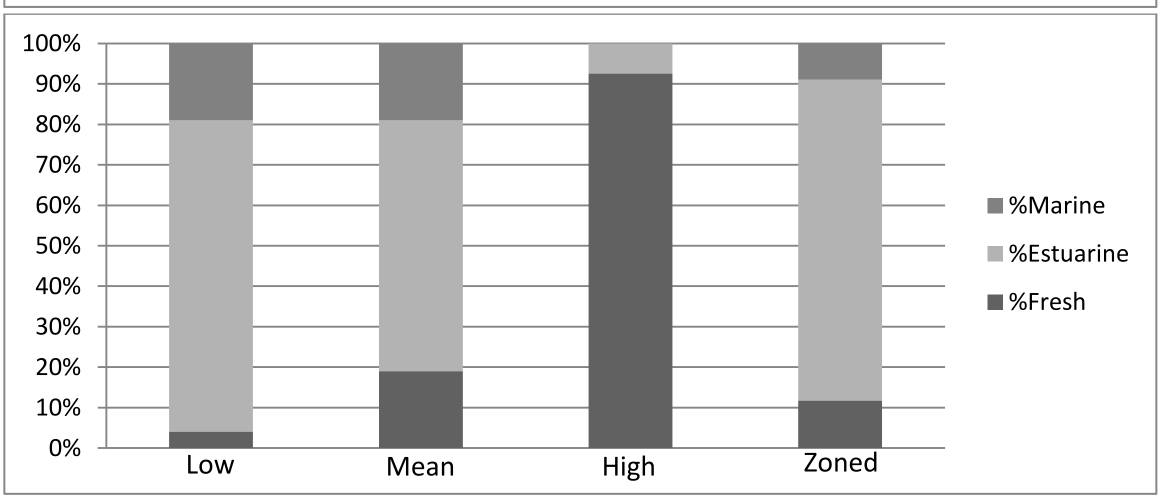
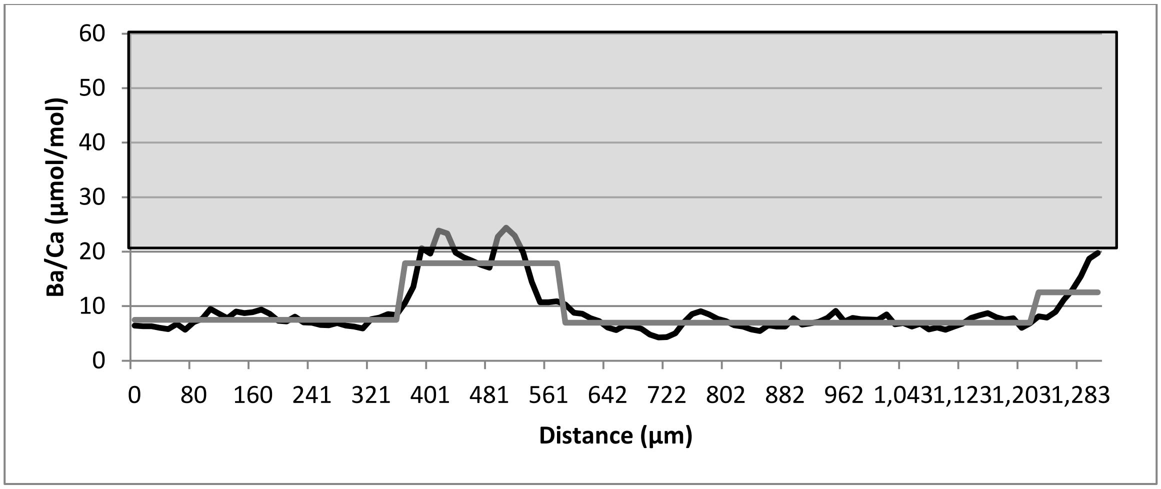
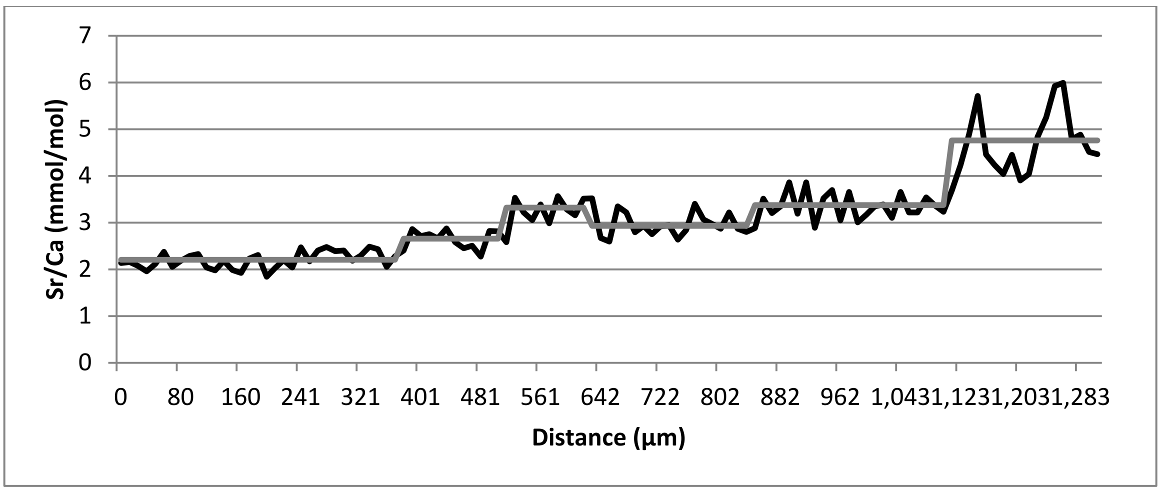


C.

Figure AB.26. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 40. Figure 26.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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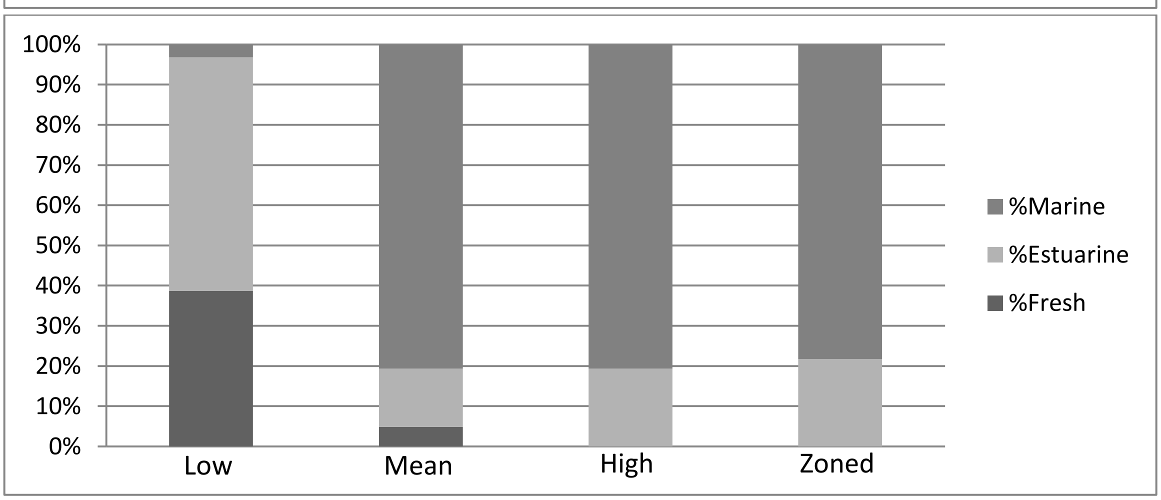
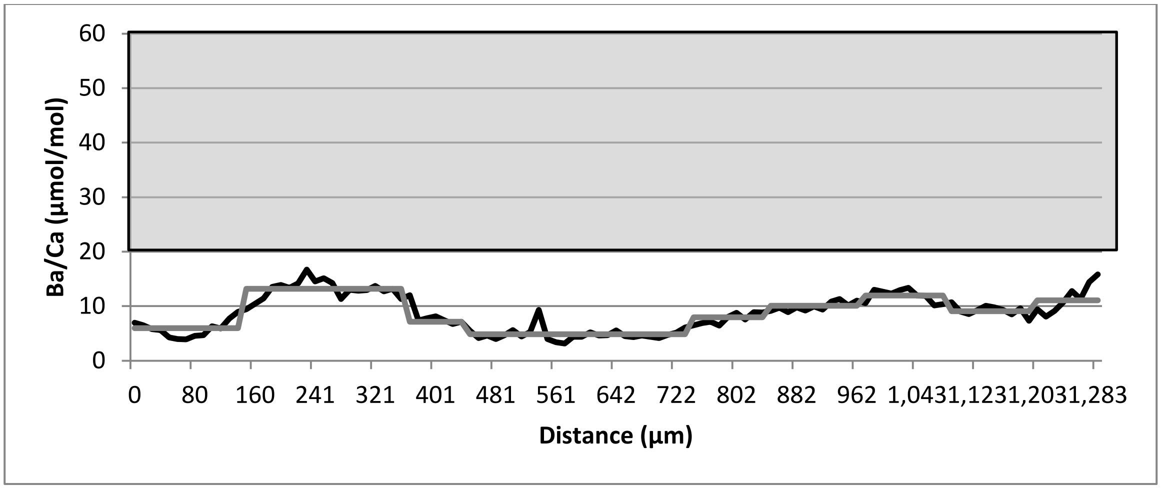
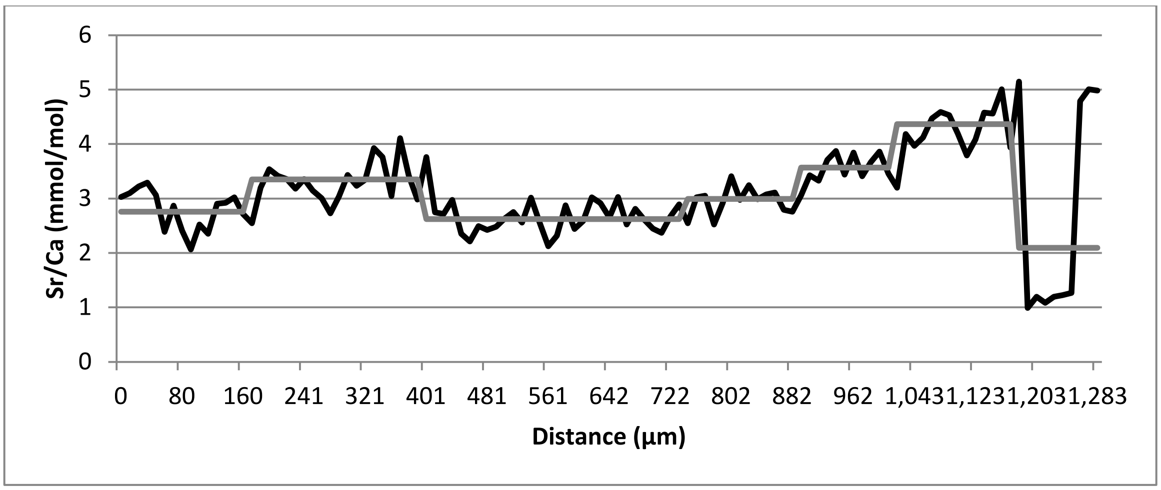


C.

Figure AB.27. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 41. Figure 27.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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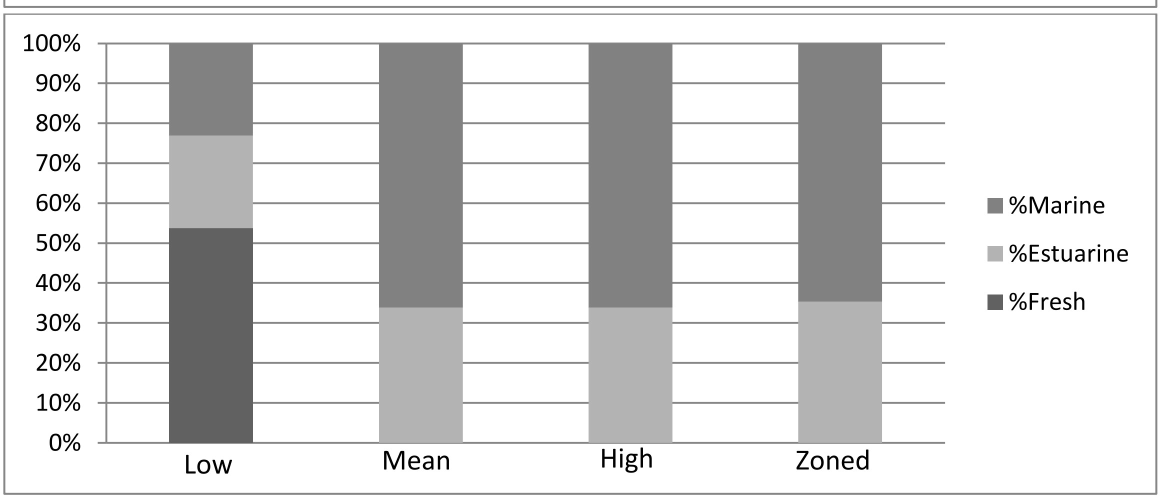
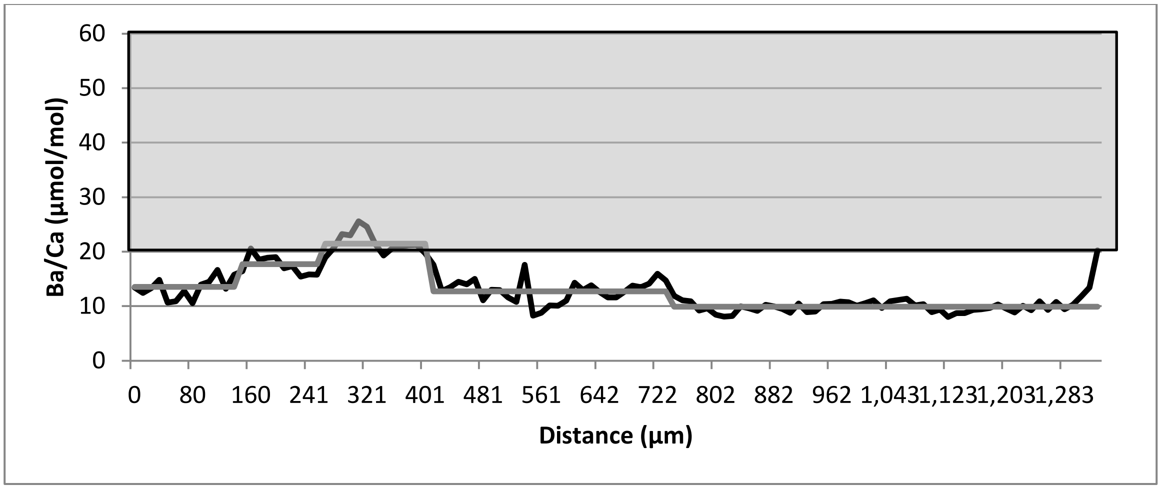


C.

Figure AB.28. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 42. Figure 28.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.



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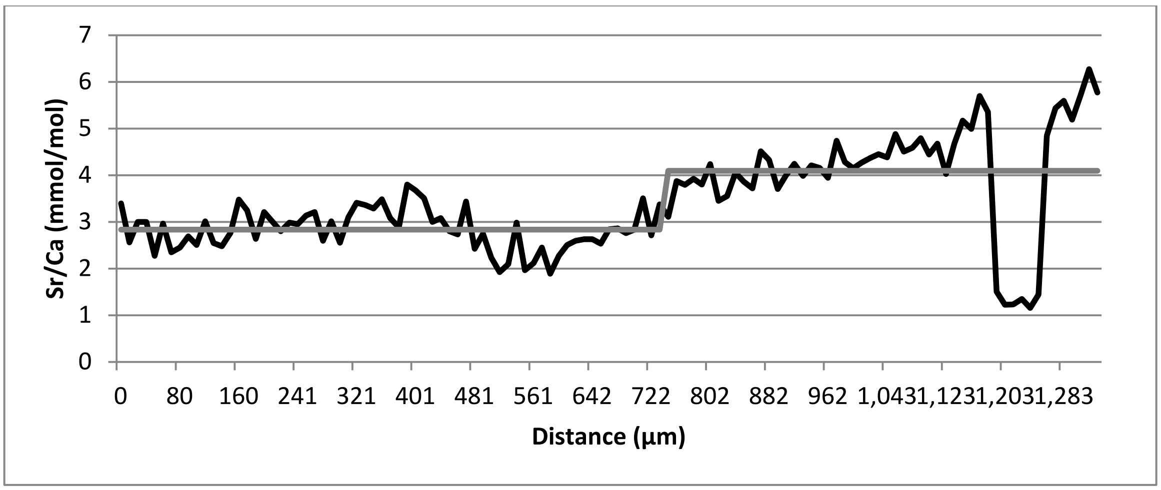


C.

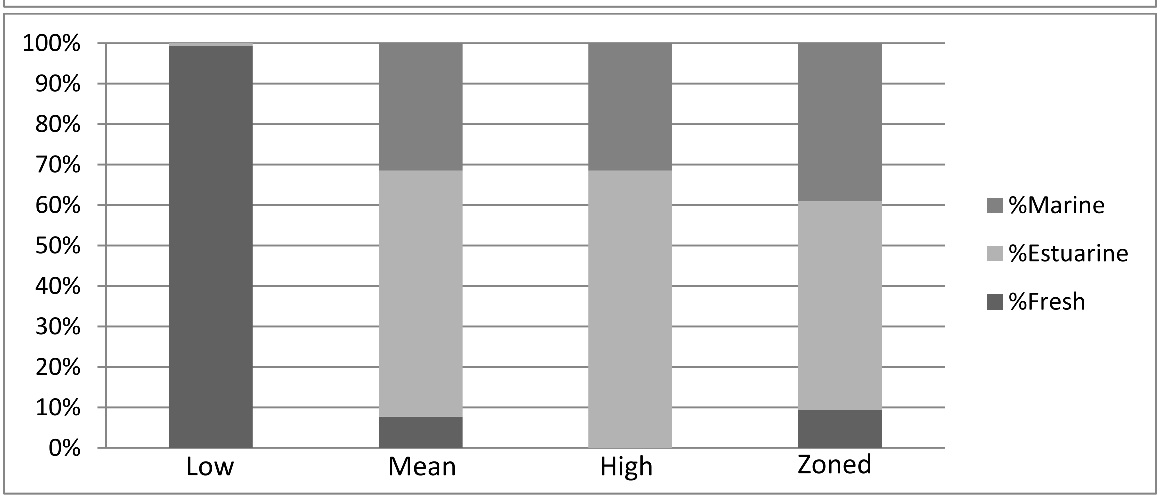


Figure AB.29. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 44. Figure 29.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

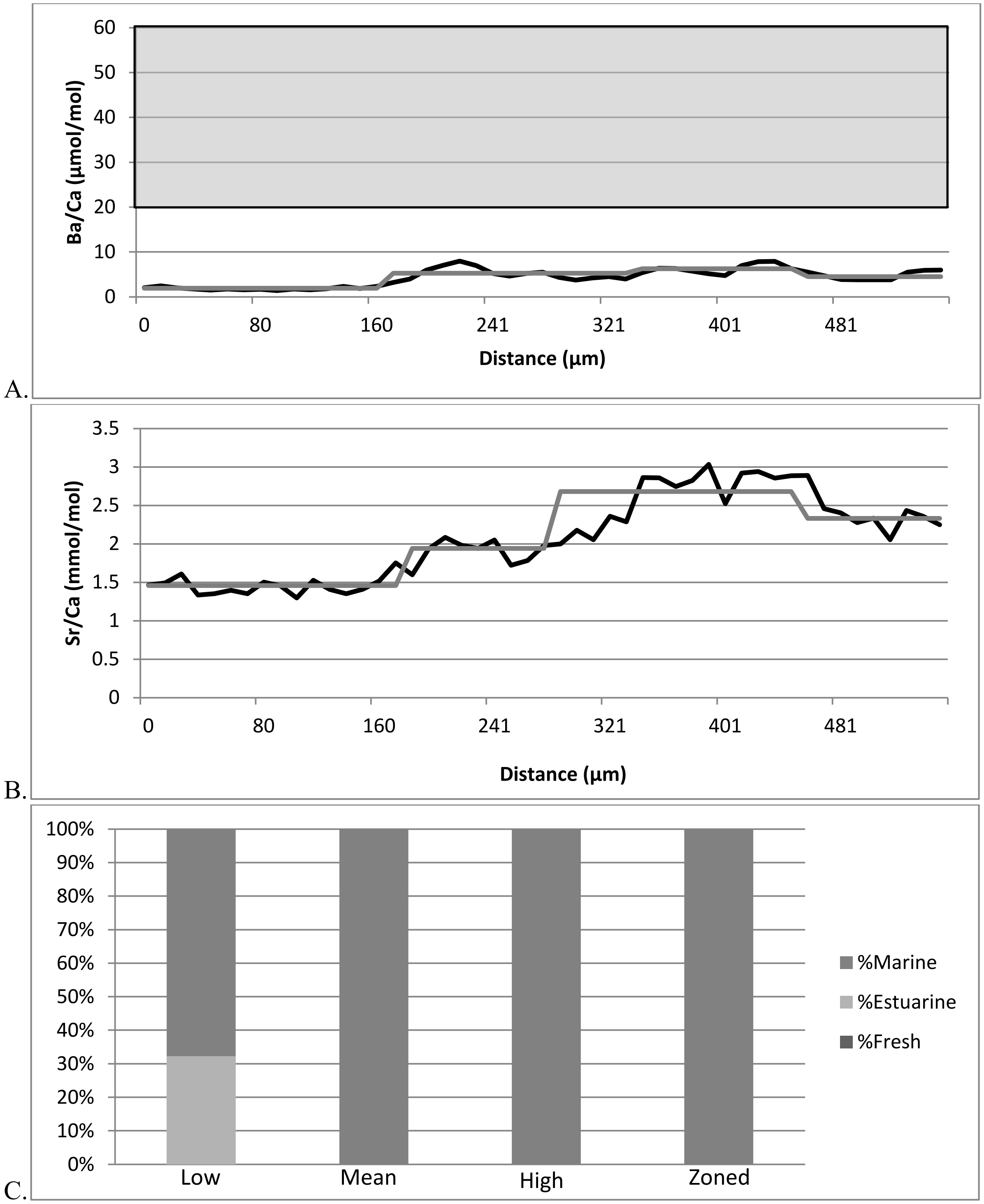
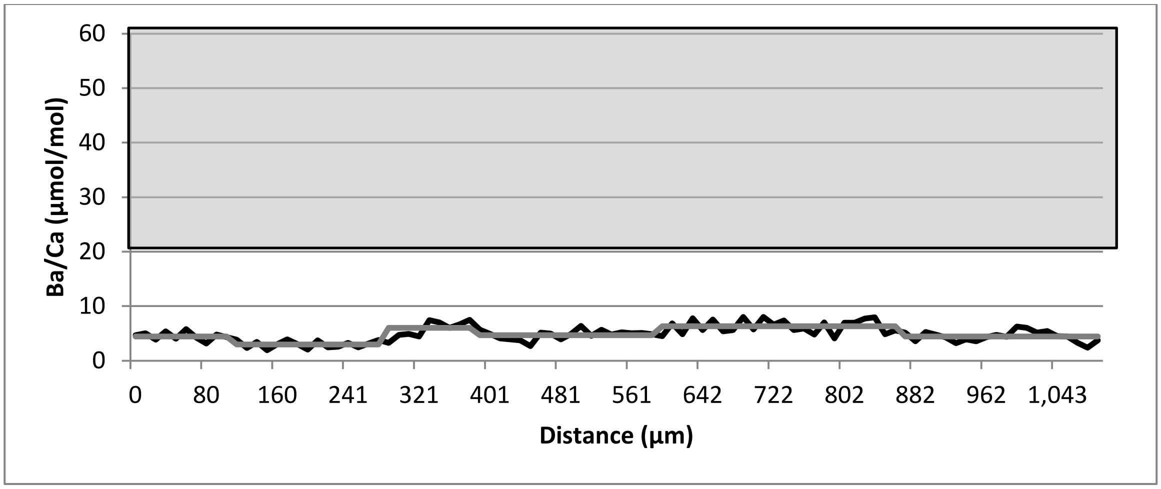
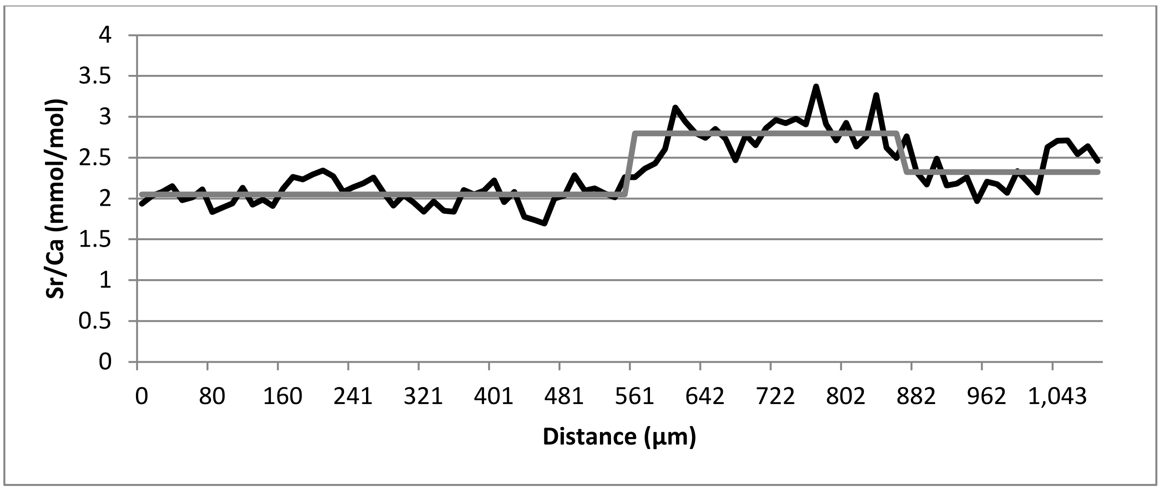


Figure AB.30. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 51. Figure 30.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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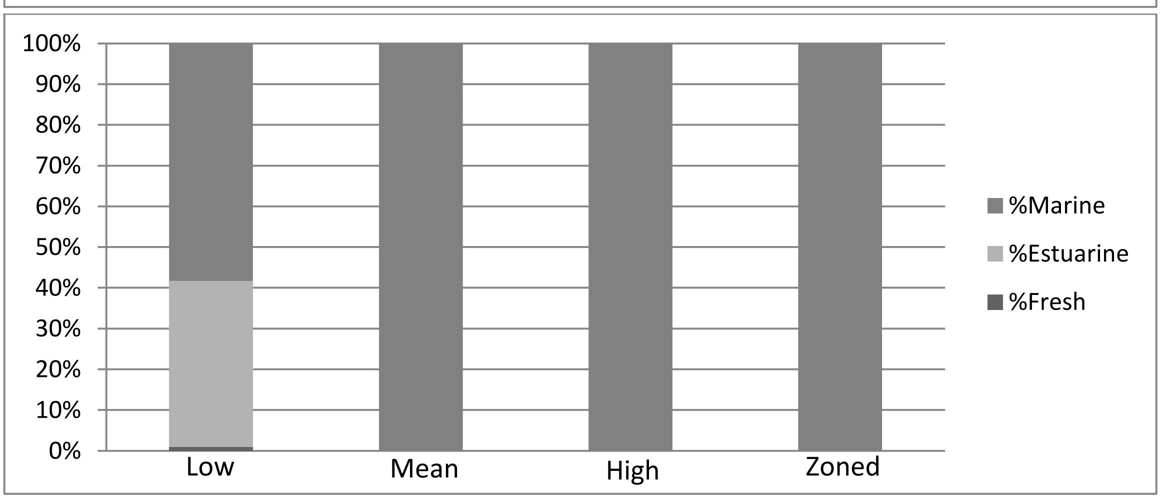
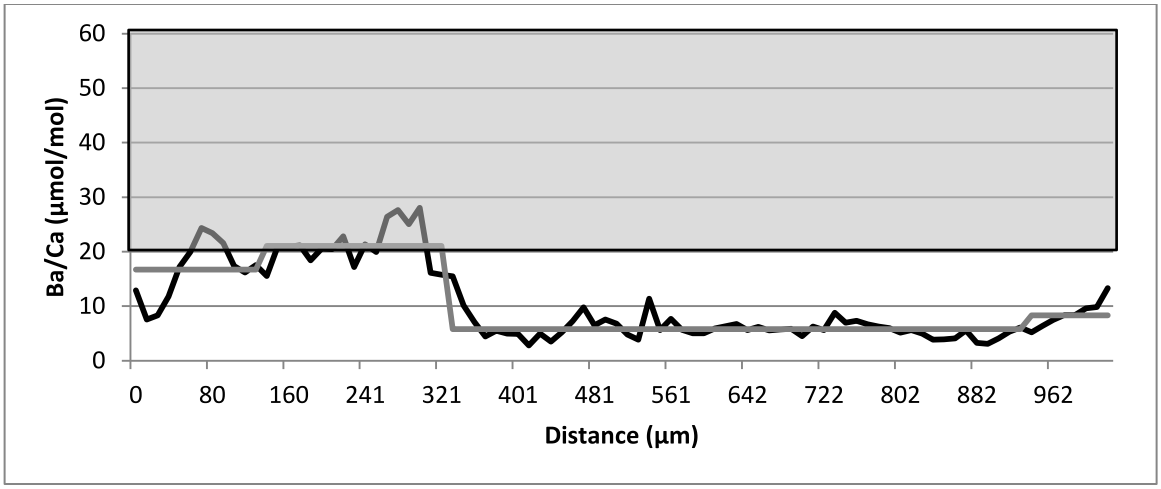
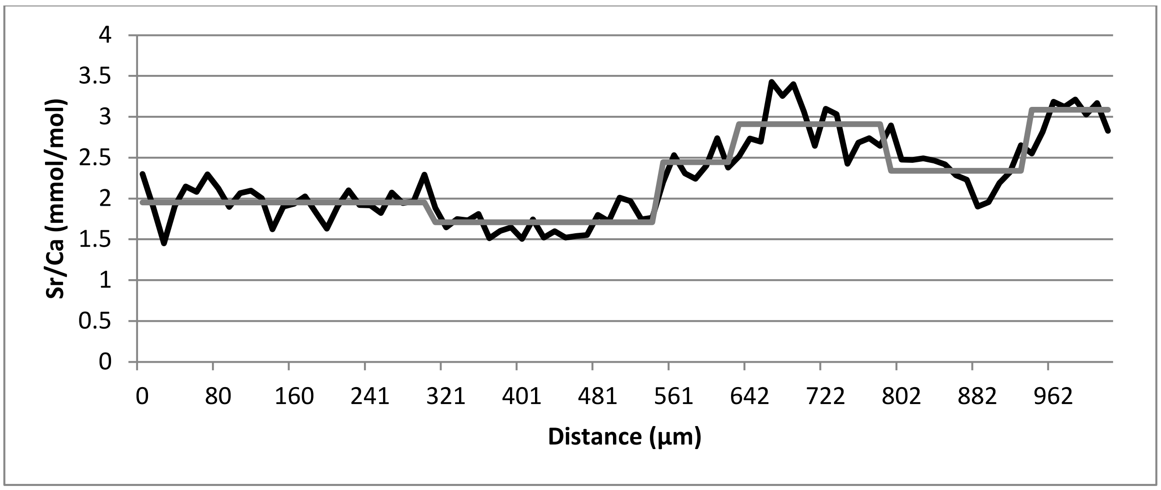


C.

Figure AB.31. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 54. Figure 31.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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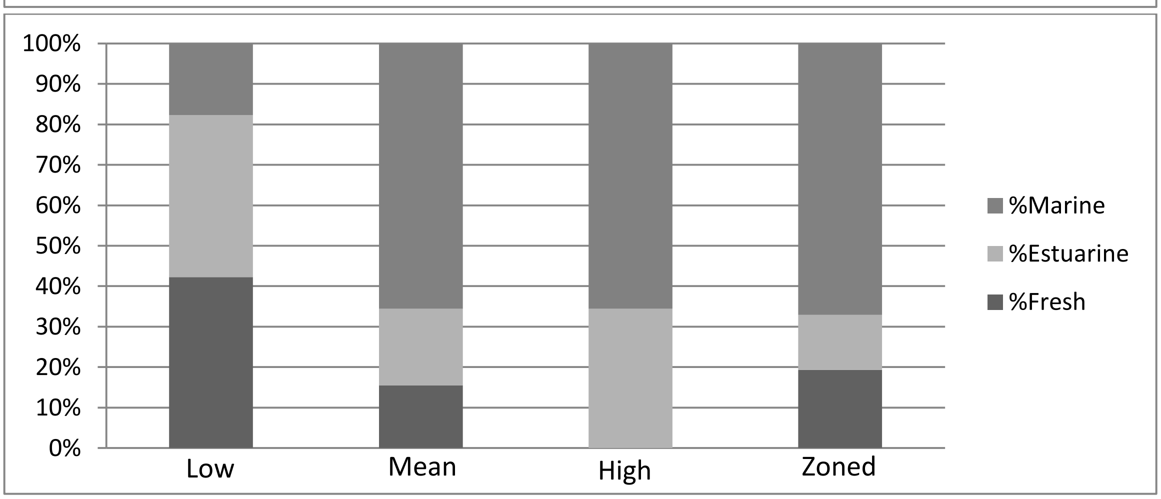
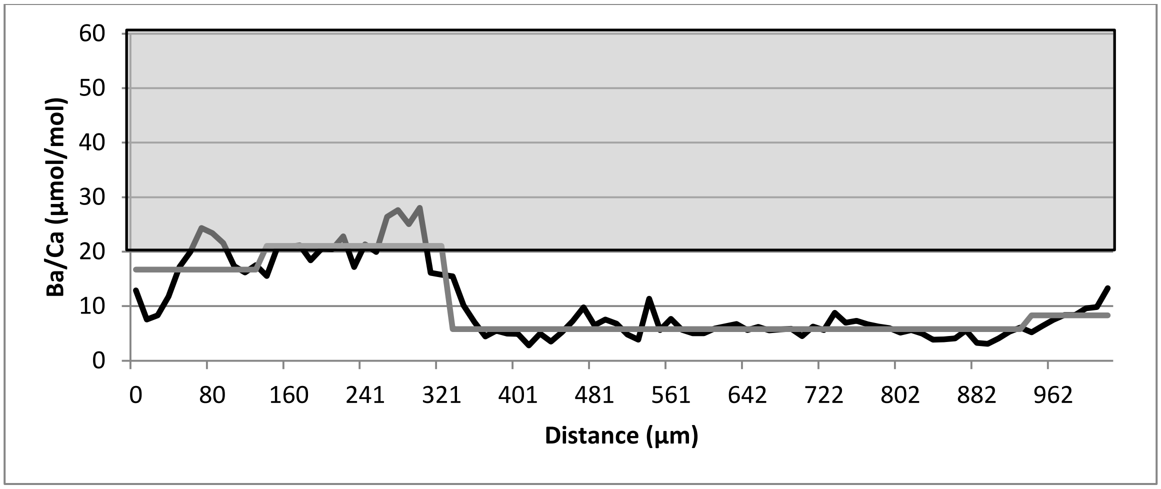
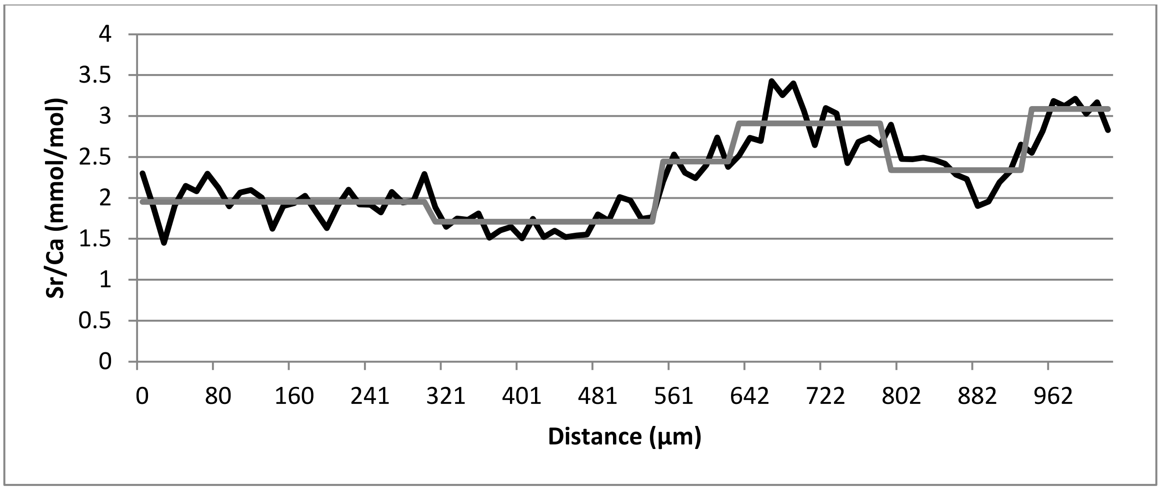


C.

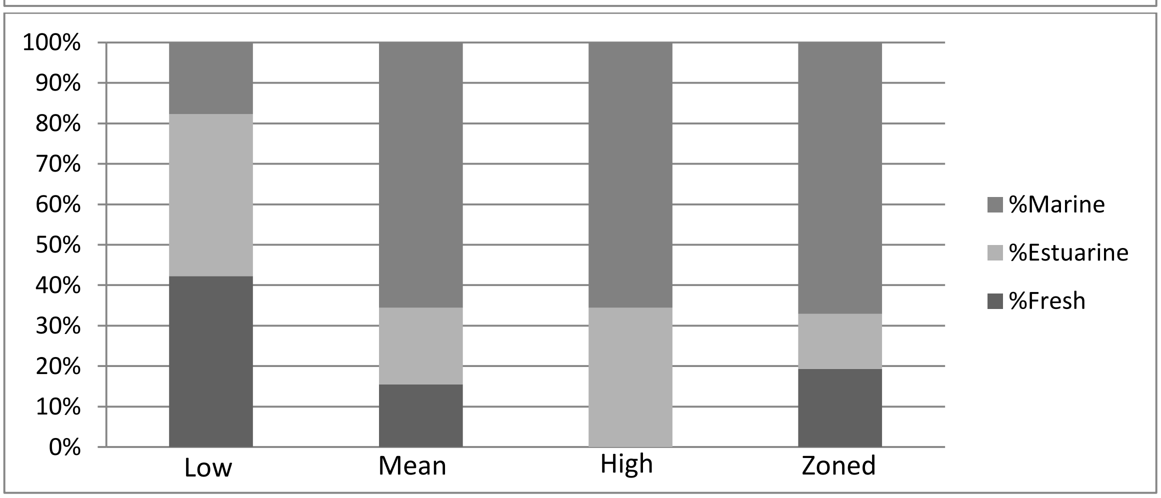
Figure AB.32. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 55. Figure 32.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.33. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 55. Figure 33.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

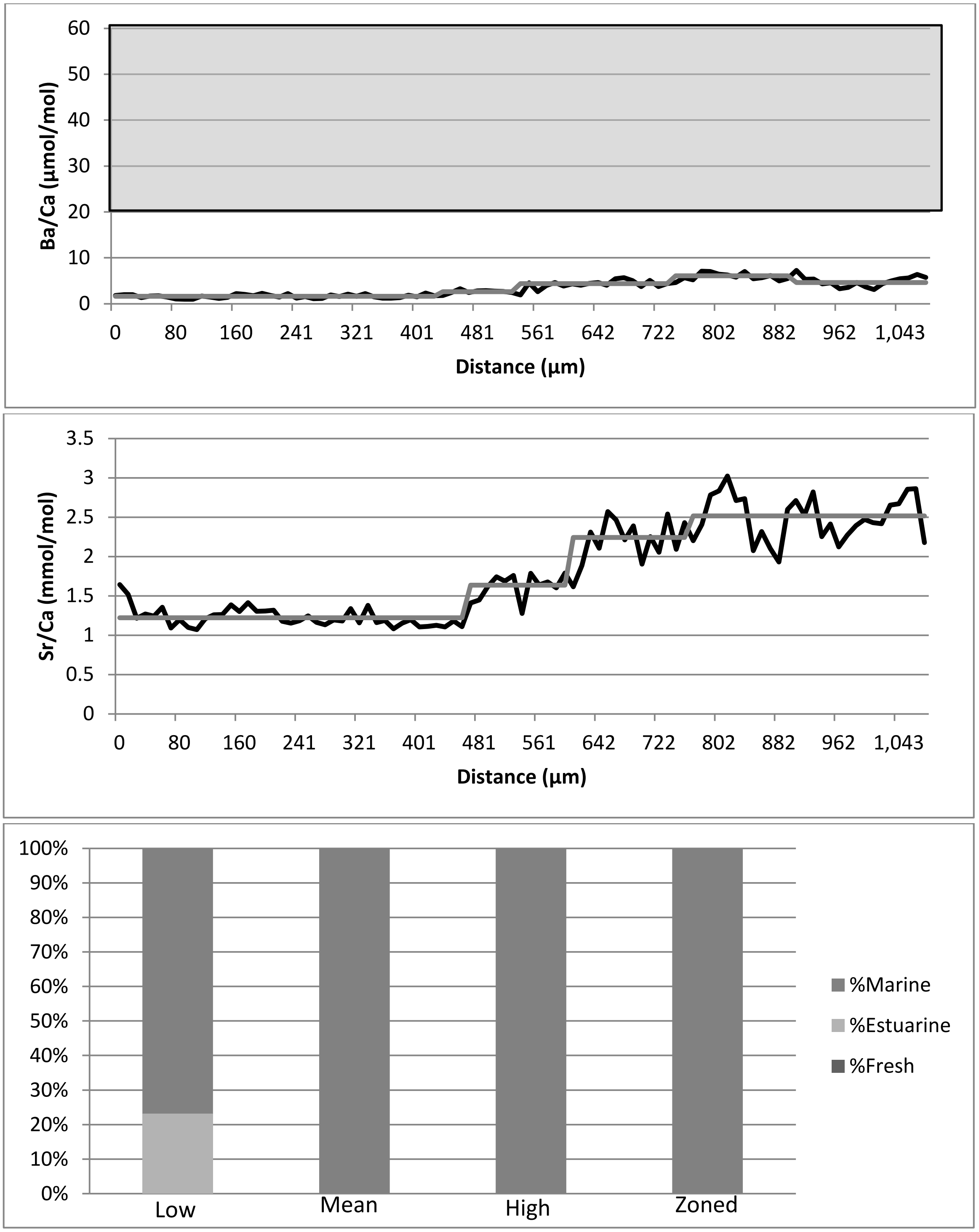
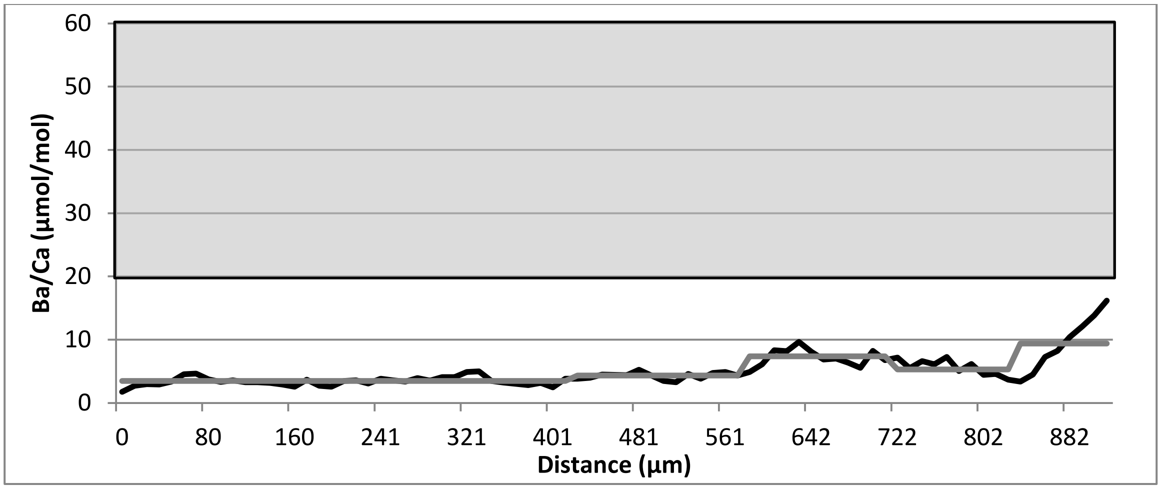
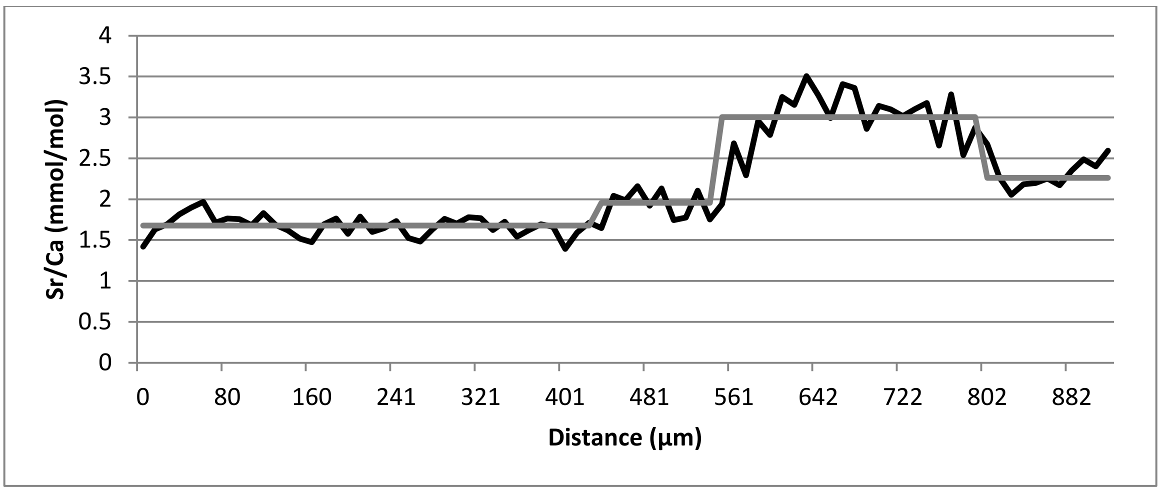


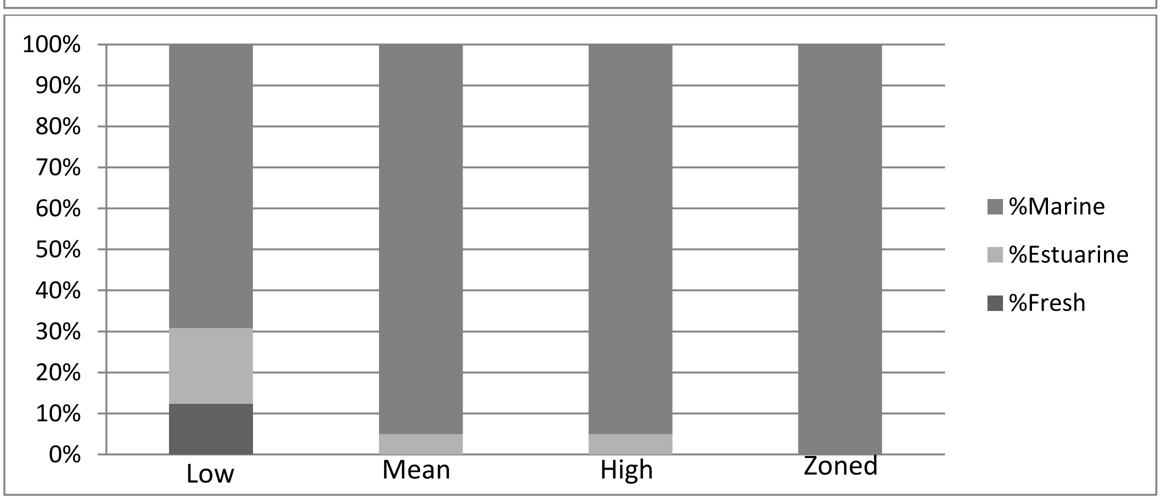
Figure AB.34. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 56. Figure 34.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

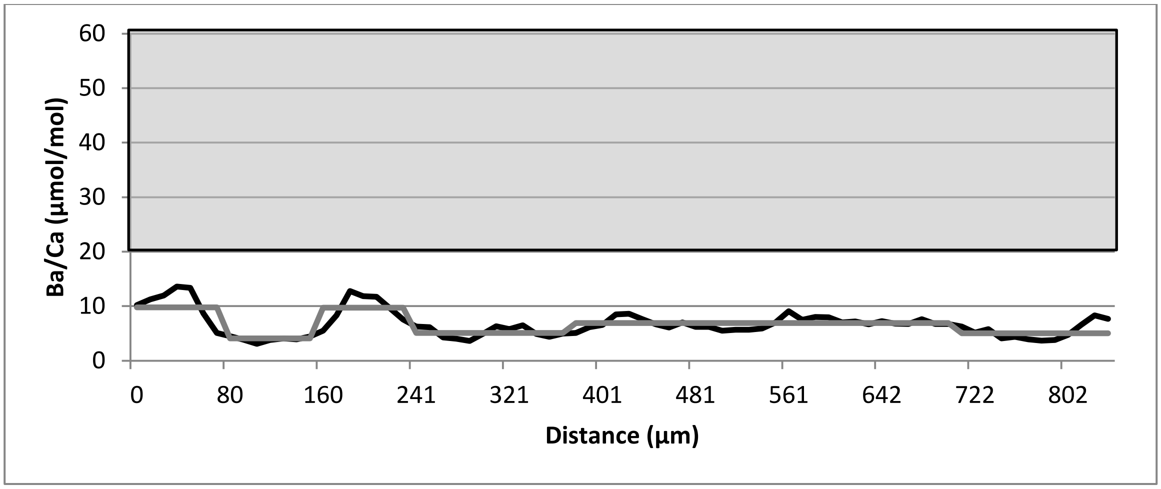


B.

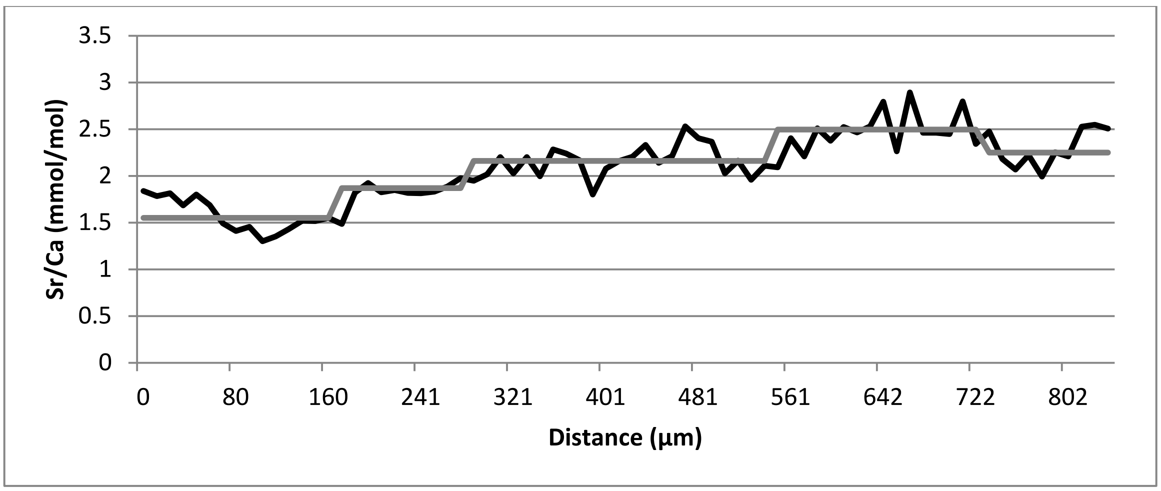


C.

Figure AB.35. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 57. Figure 35.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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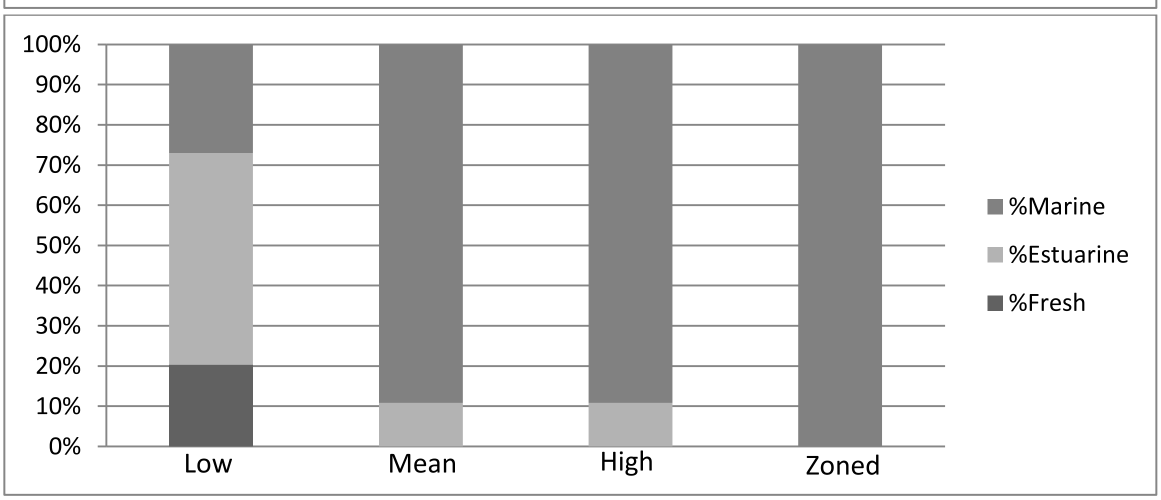
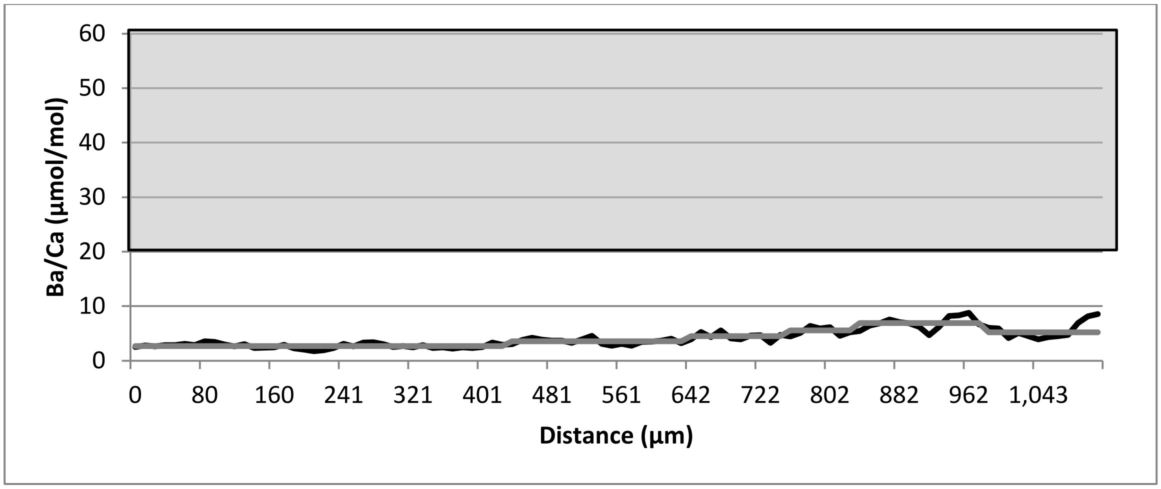
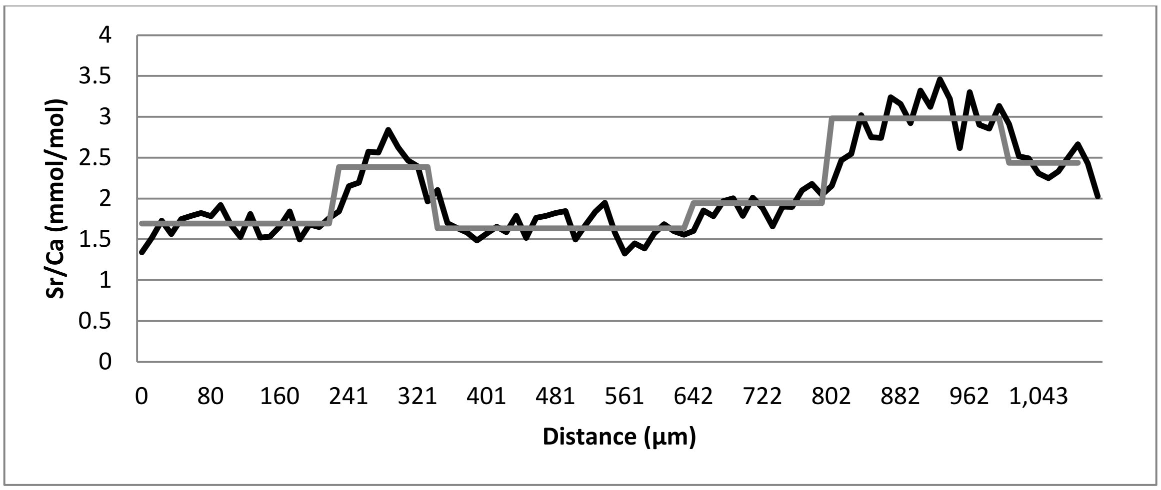


C.

Figure AB.36. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 58. Figure 36.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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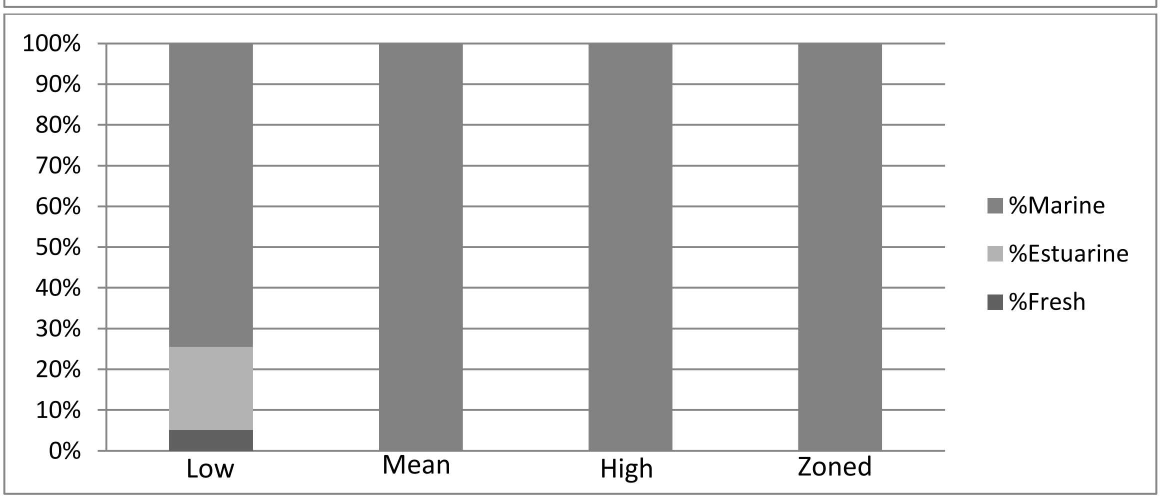
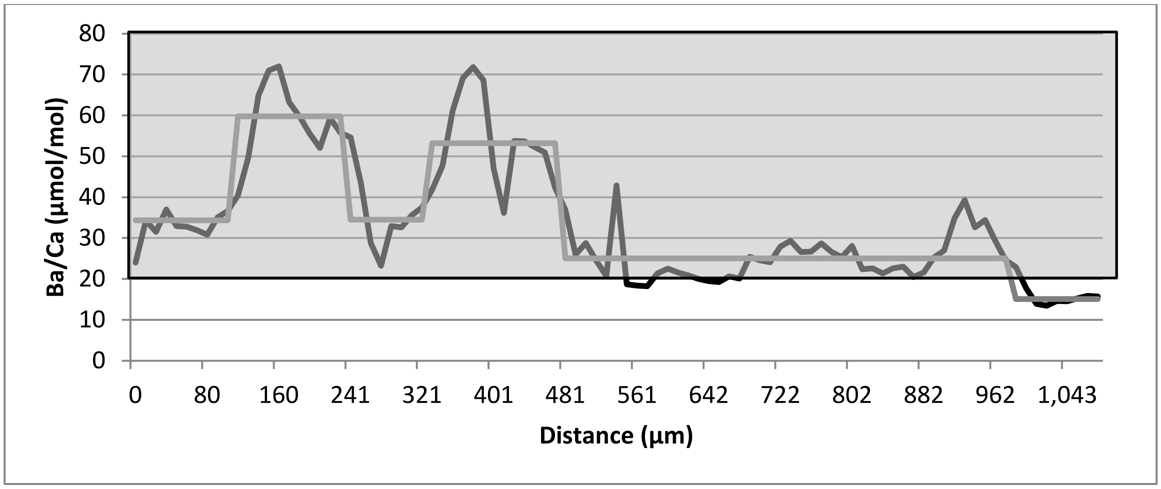
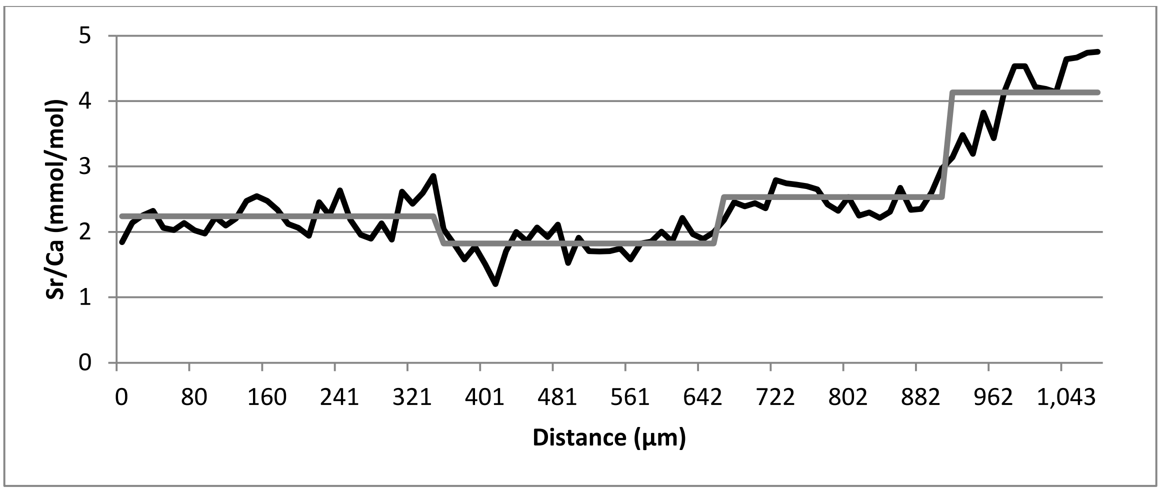


C.

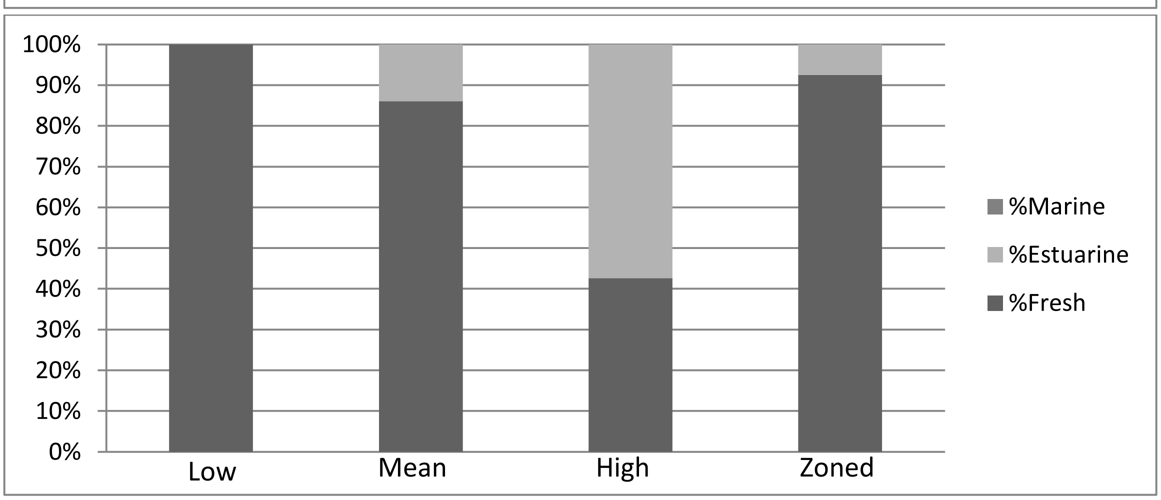
Figure AB.37. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 59. Figure 37.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

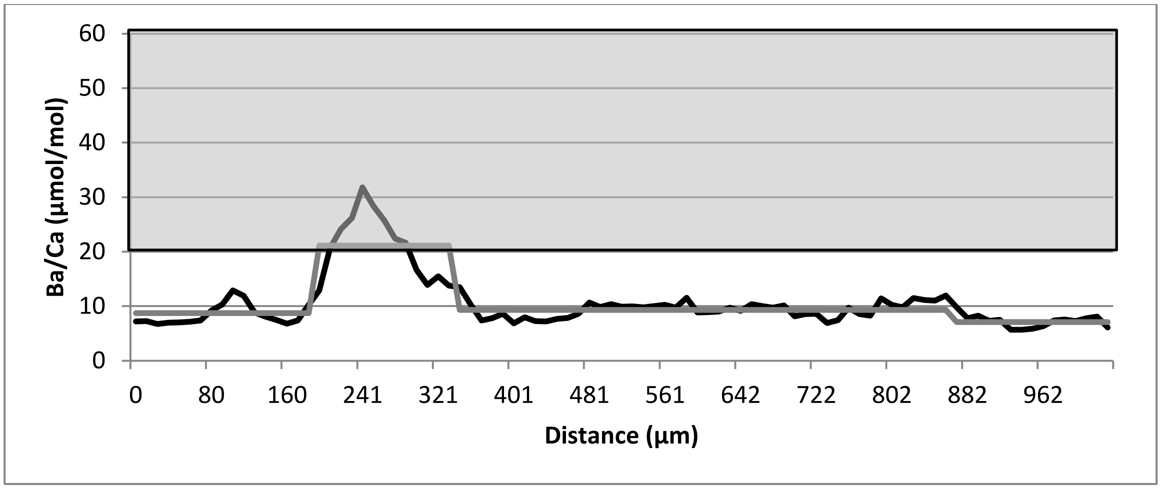


B.



C.

Figure AB.38. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 62. Figure 38.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


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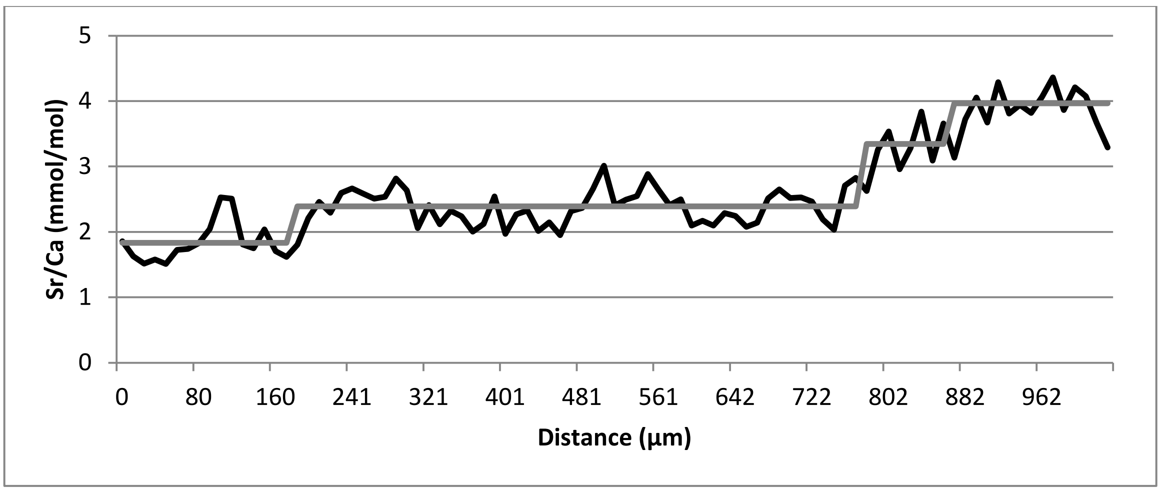


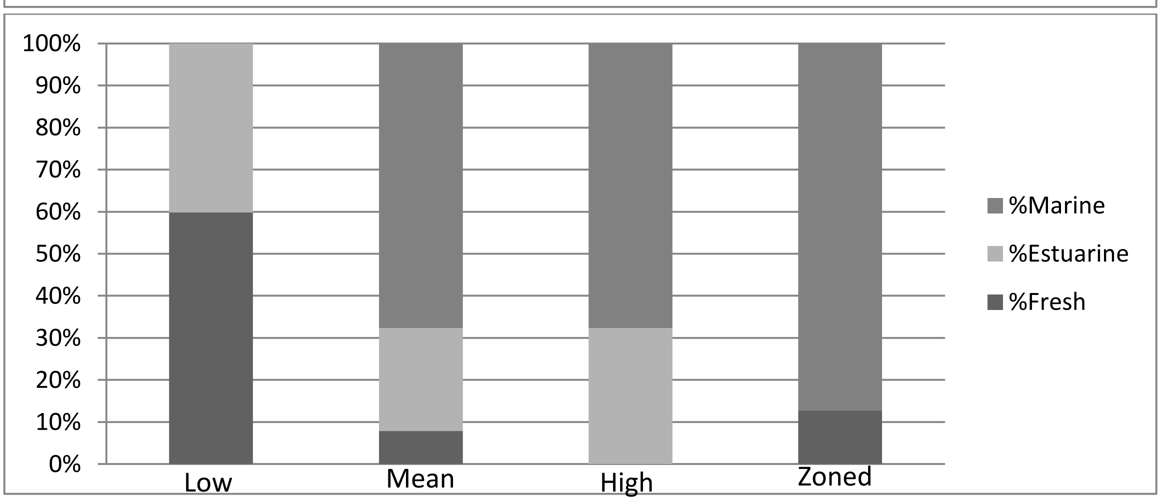
C.


Figure AB.39. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 63. Figure 39.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

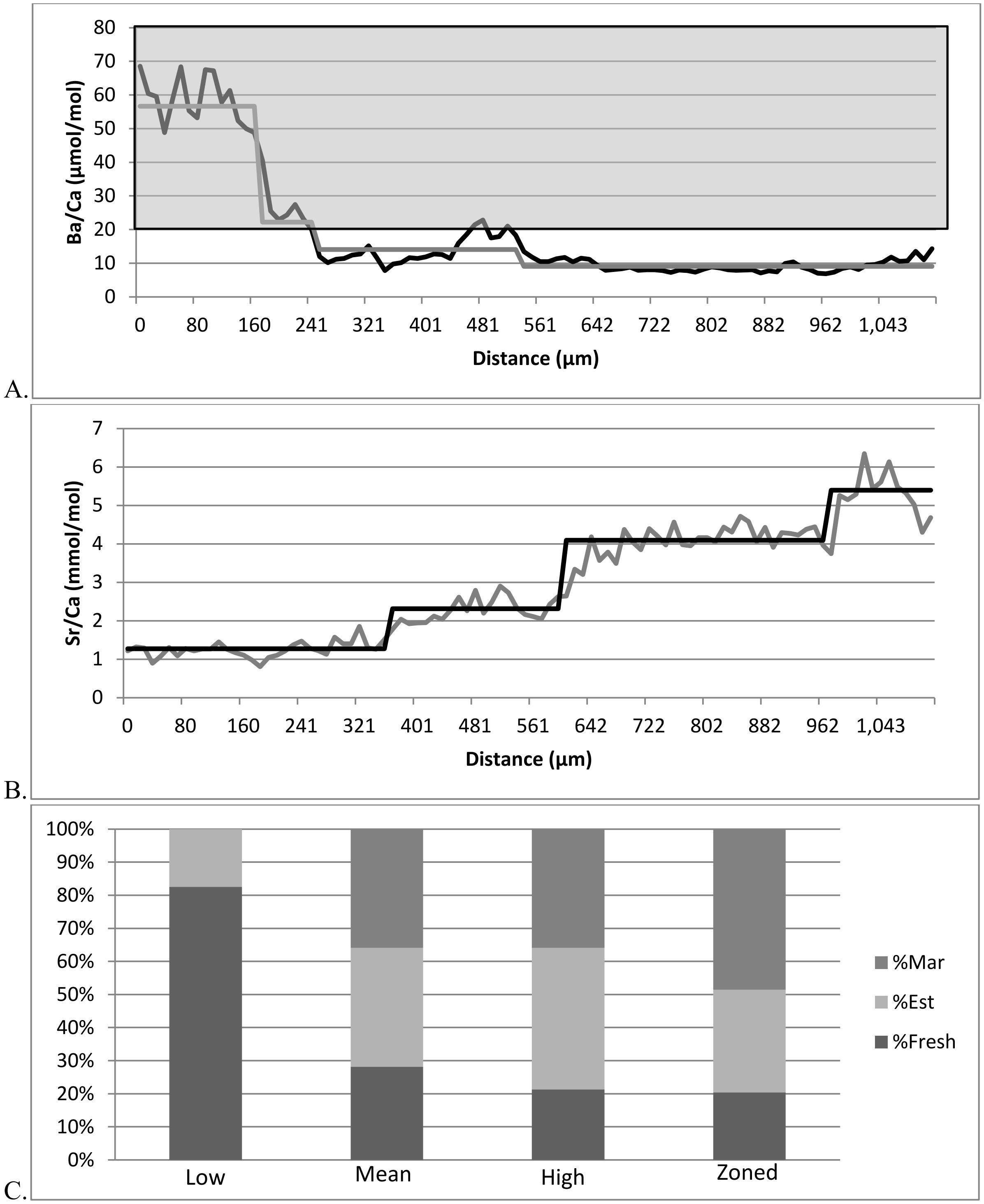
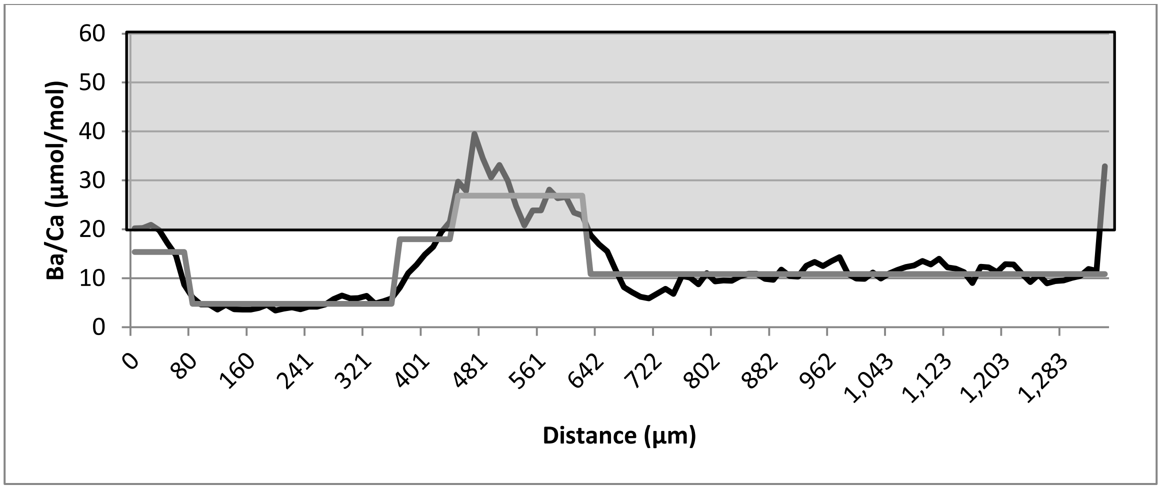
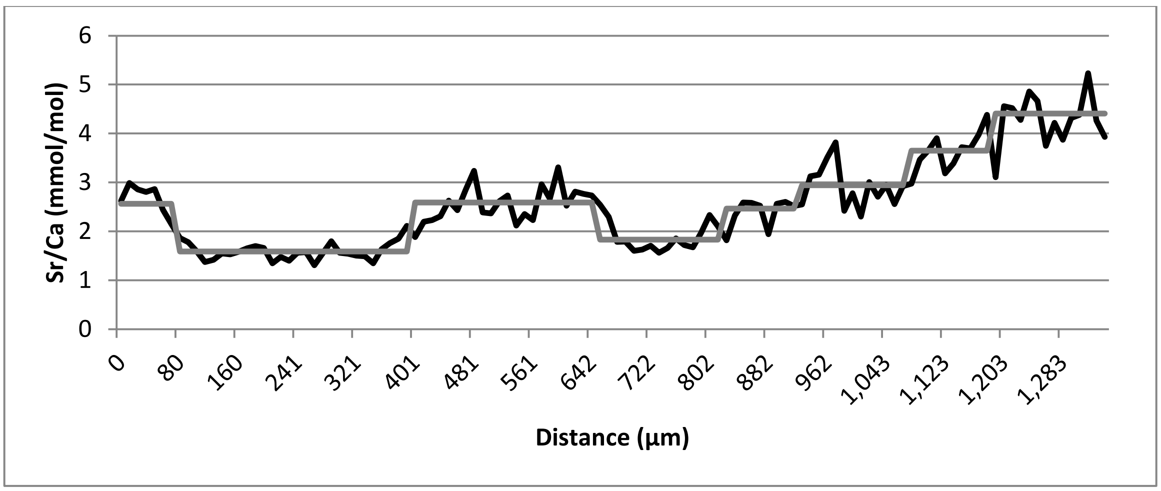


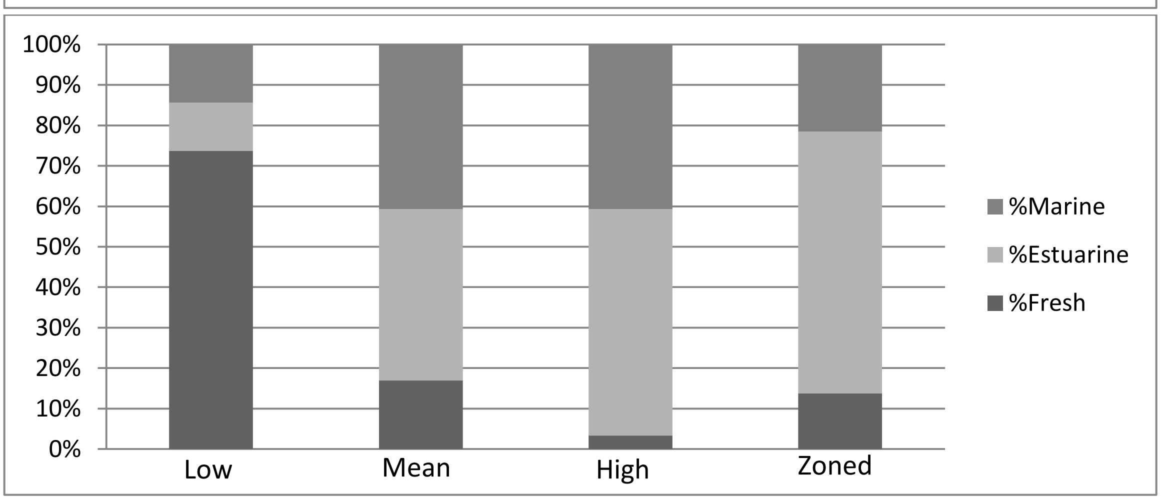
Figure AB.40. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 64. Figure 40.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.41. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 65. Figure 41.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

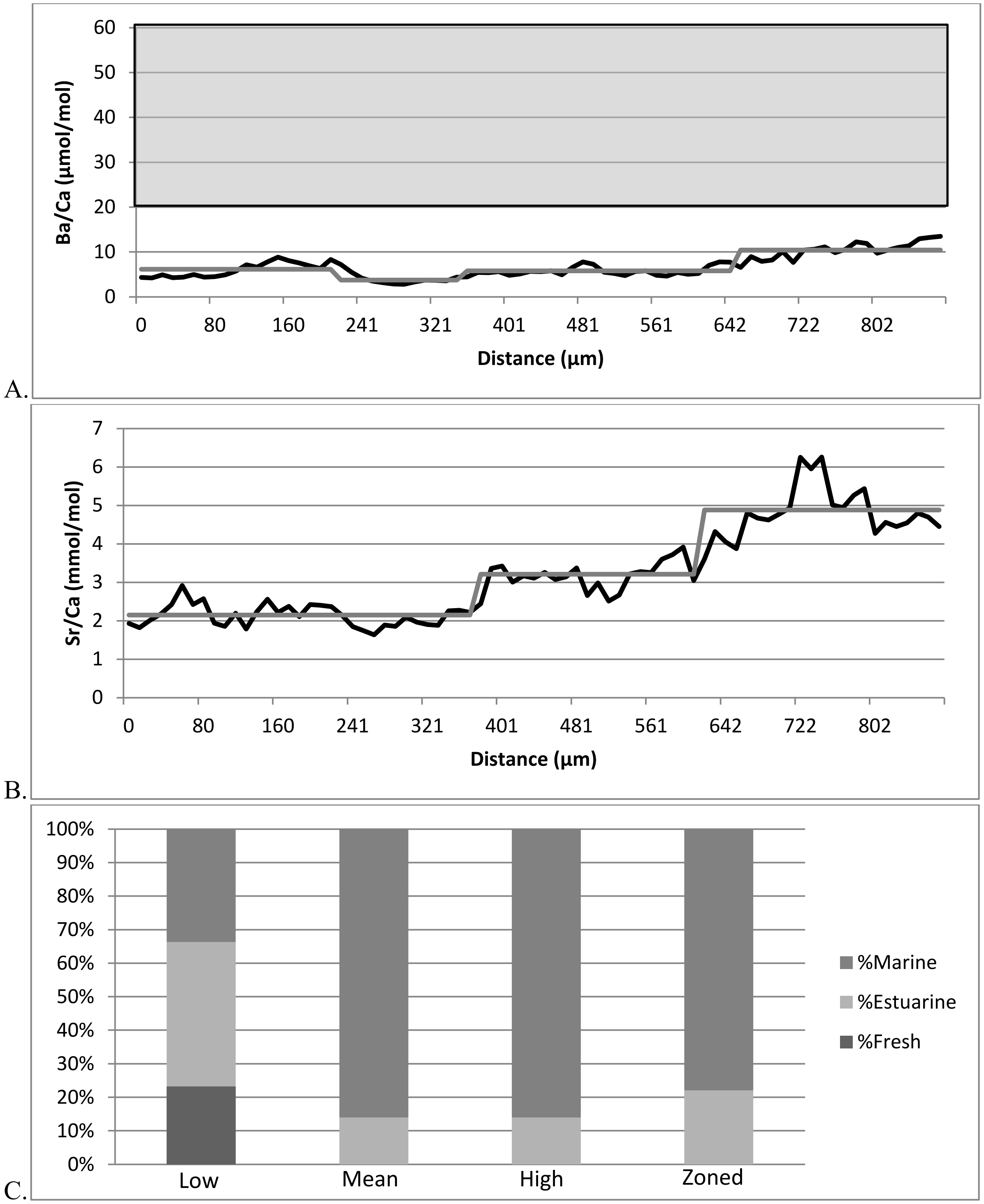
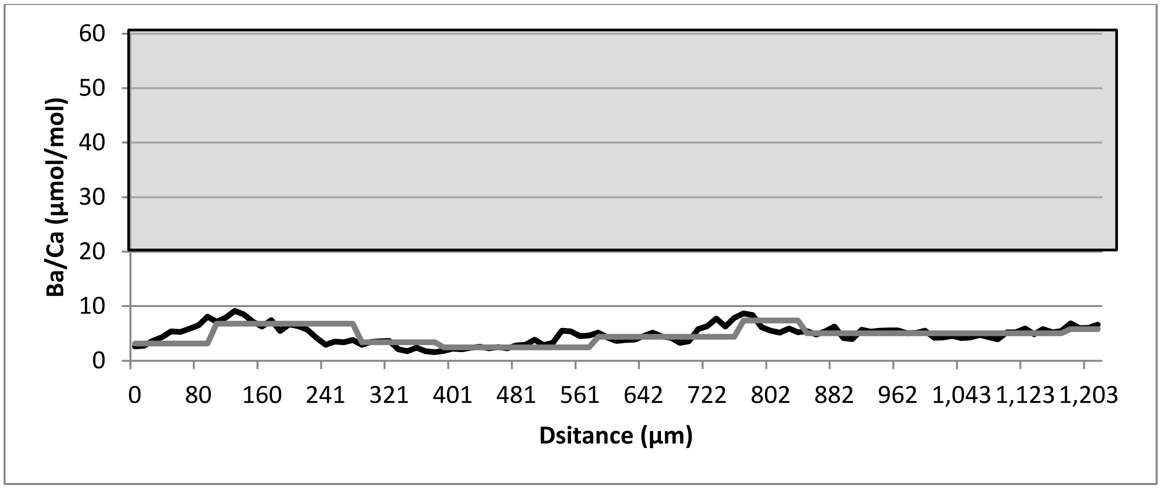
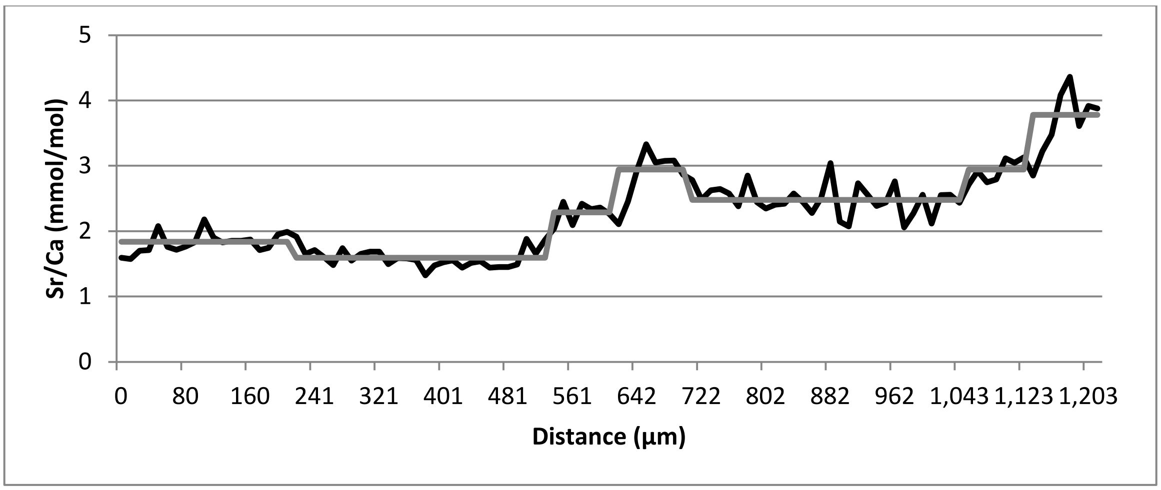


Figure AB.42. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 66. Figure 42.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


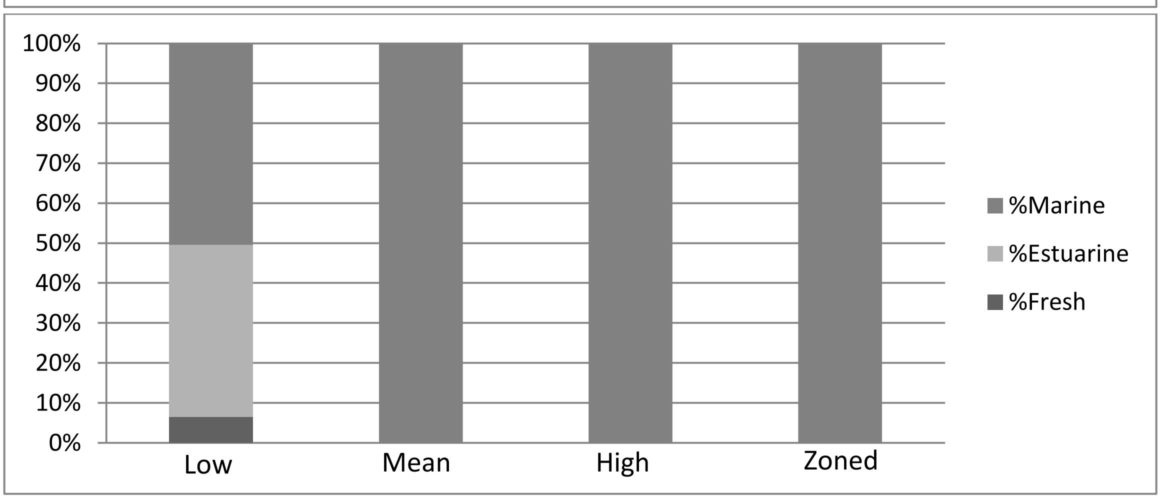
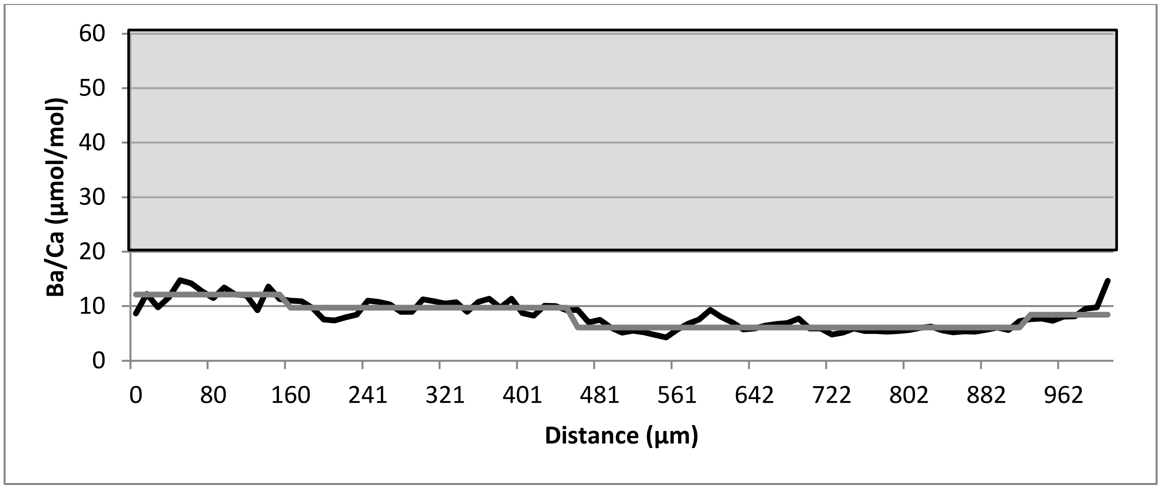
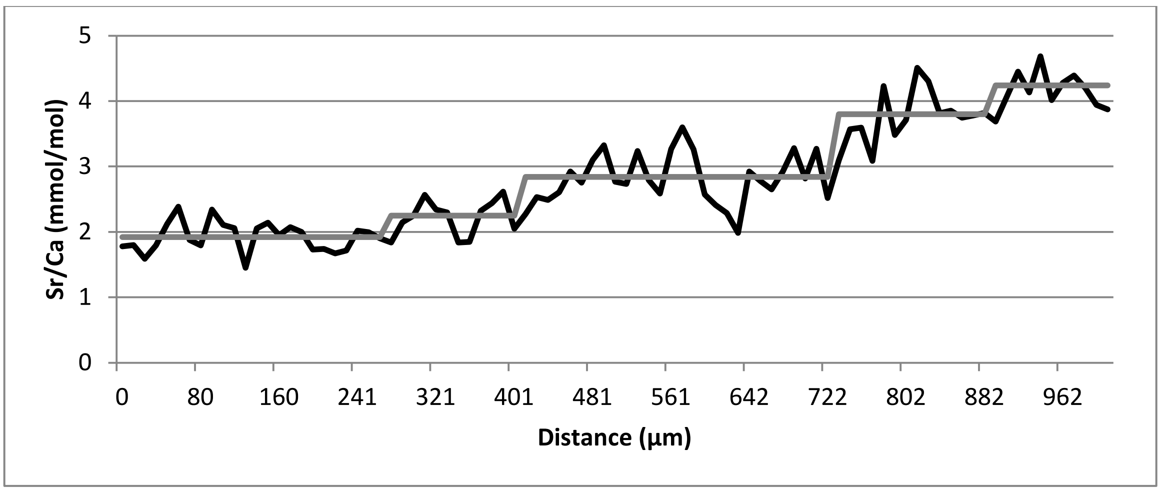
C.


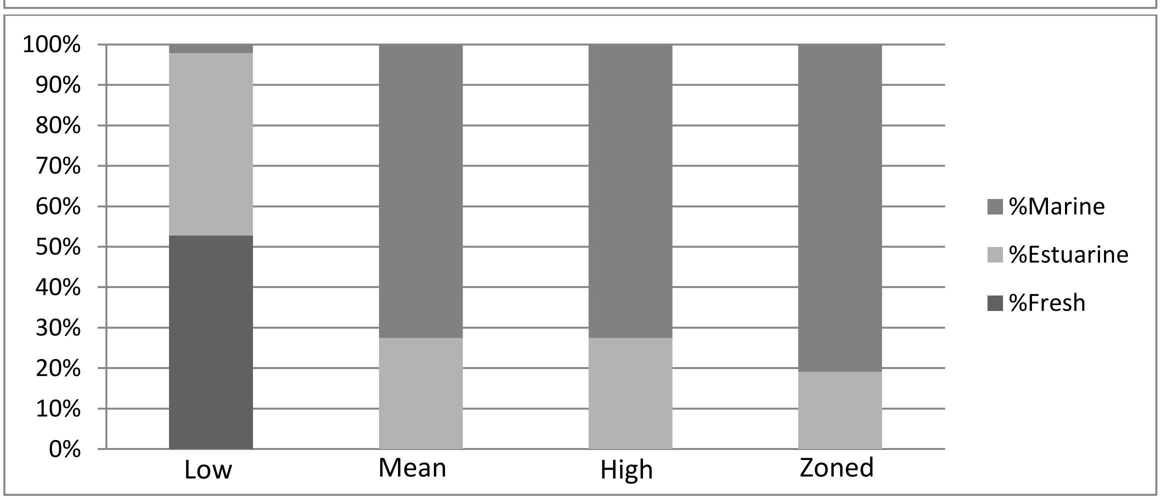
Figure AB.43. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 68. Figure 43.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

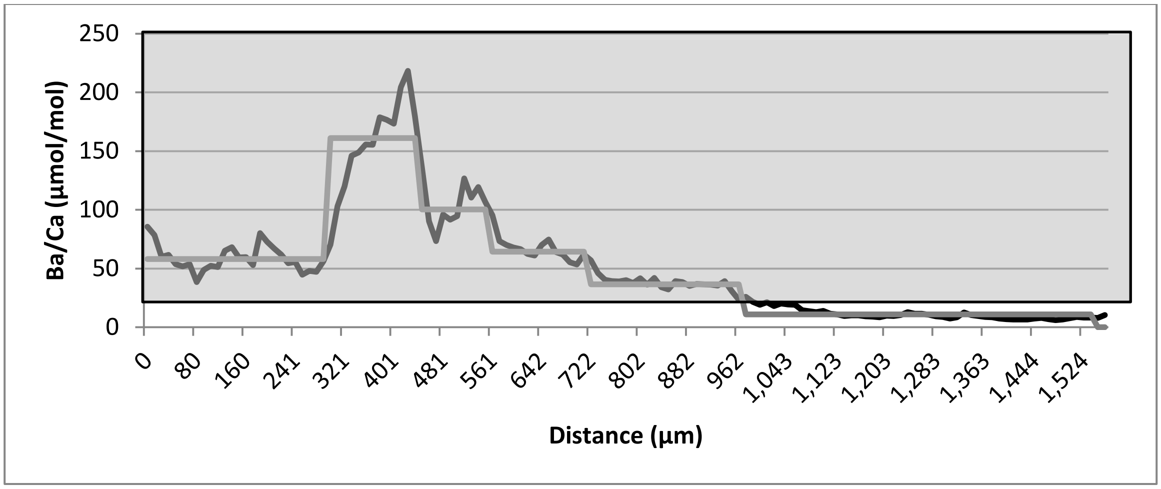


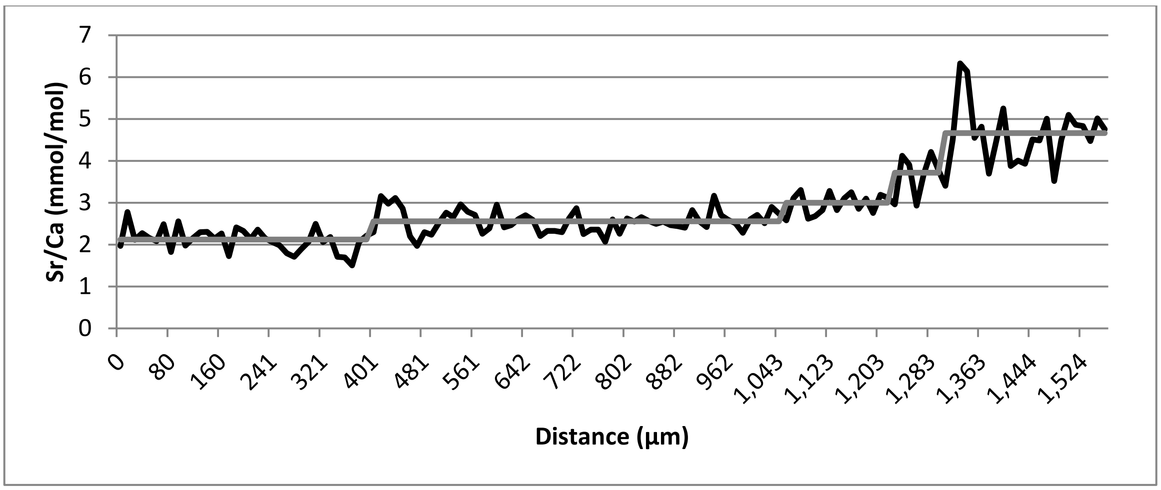
B.



C.

Figure AB.44. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 69. Figure 44.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A. 

B. 

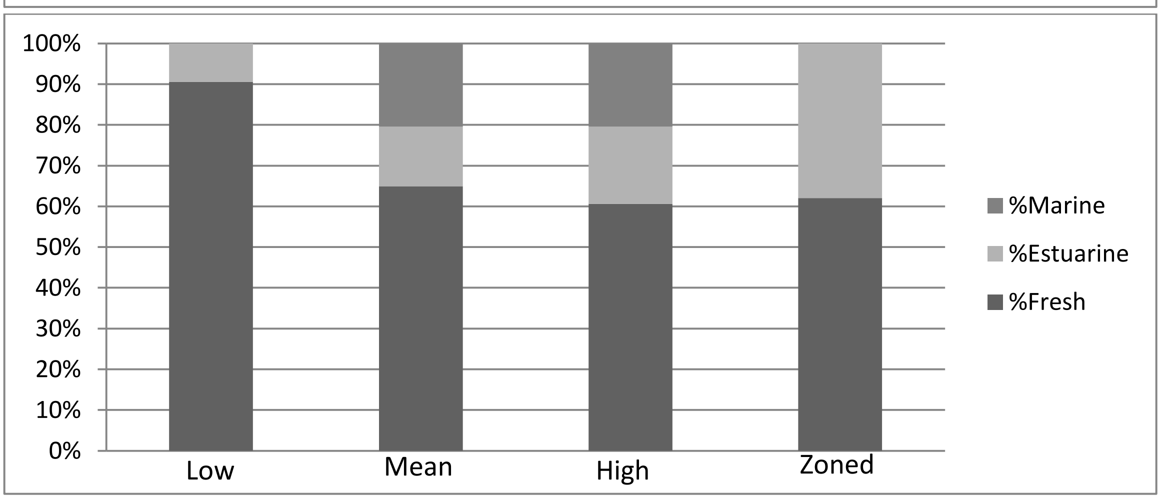
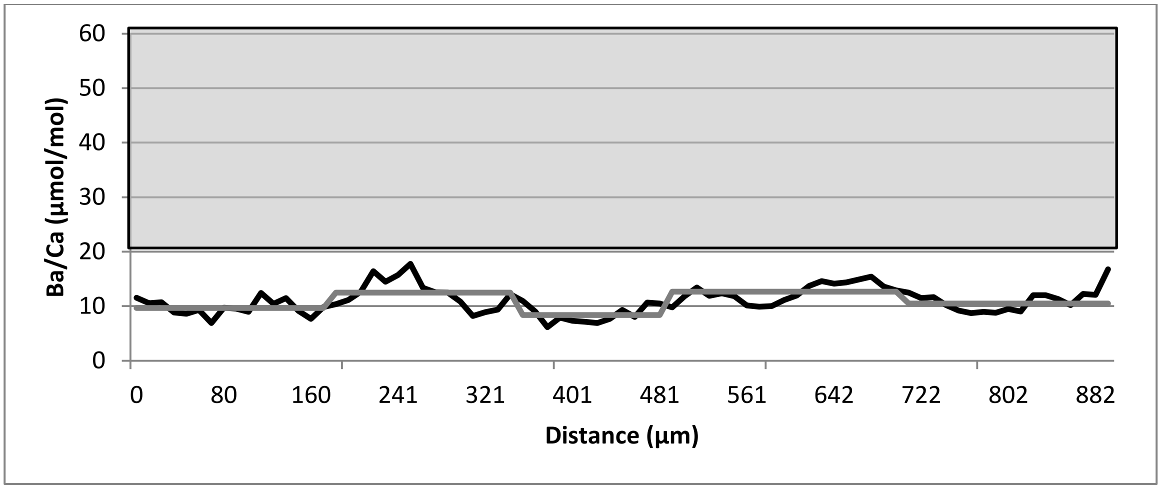
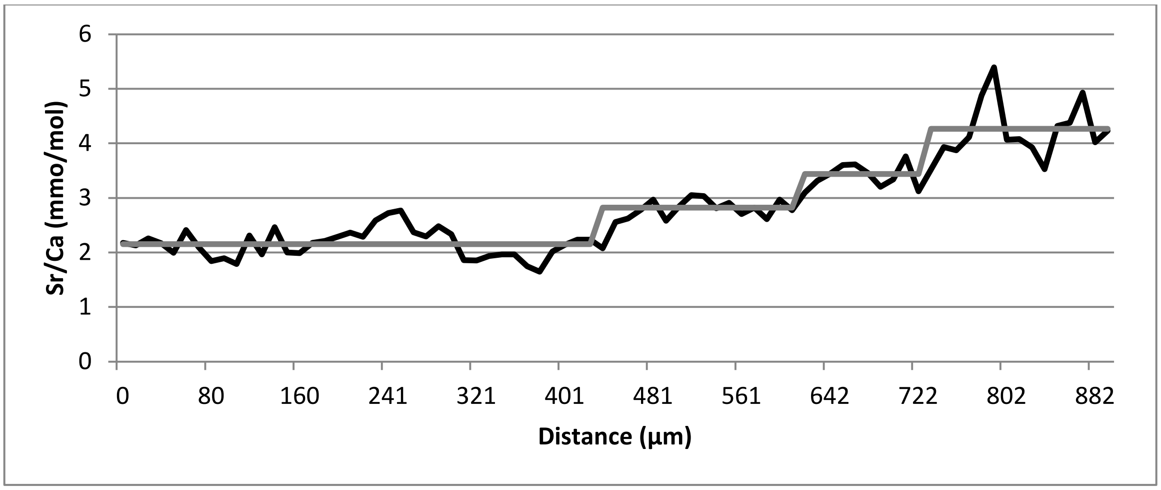
C. 

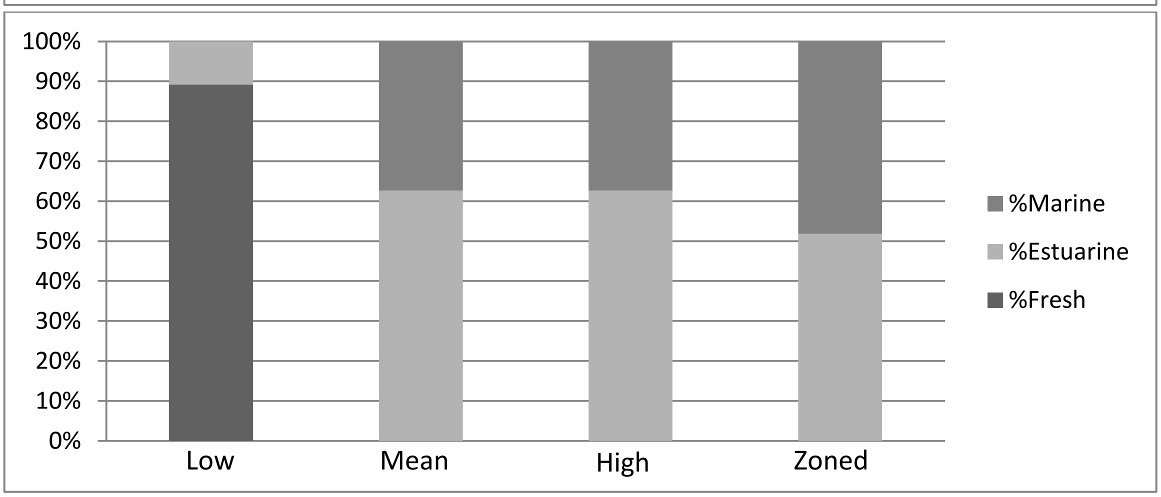
Figure AB.45. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 70. Figure 45.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.46. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 71. Figure 46.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

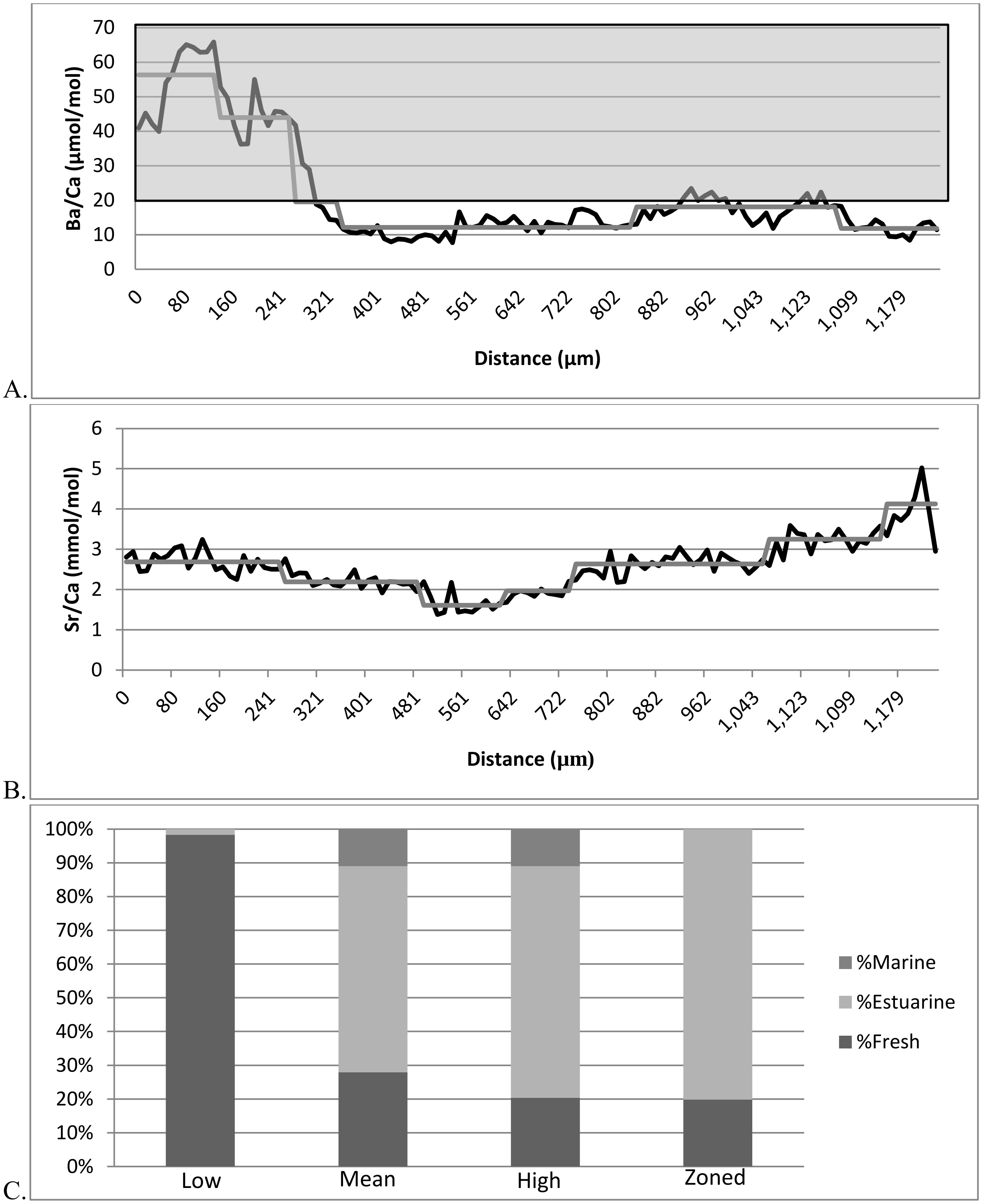
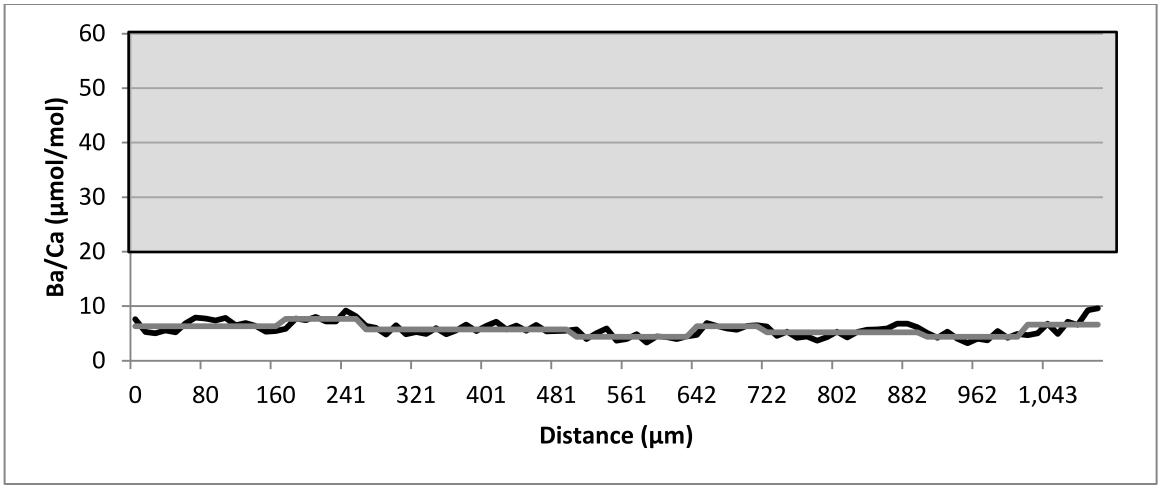
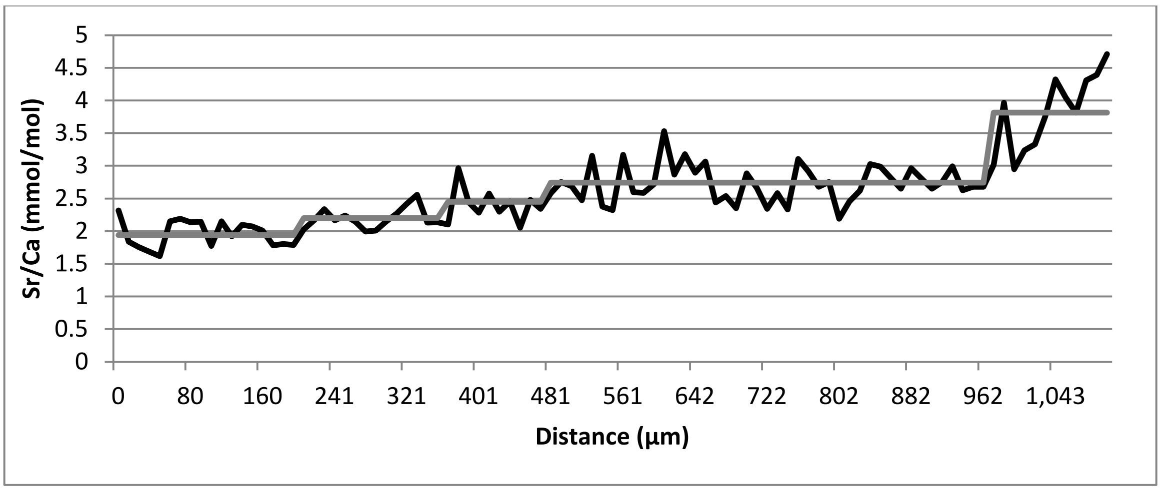


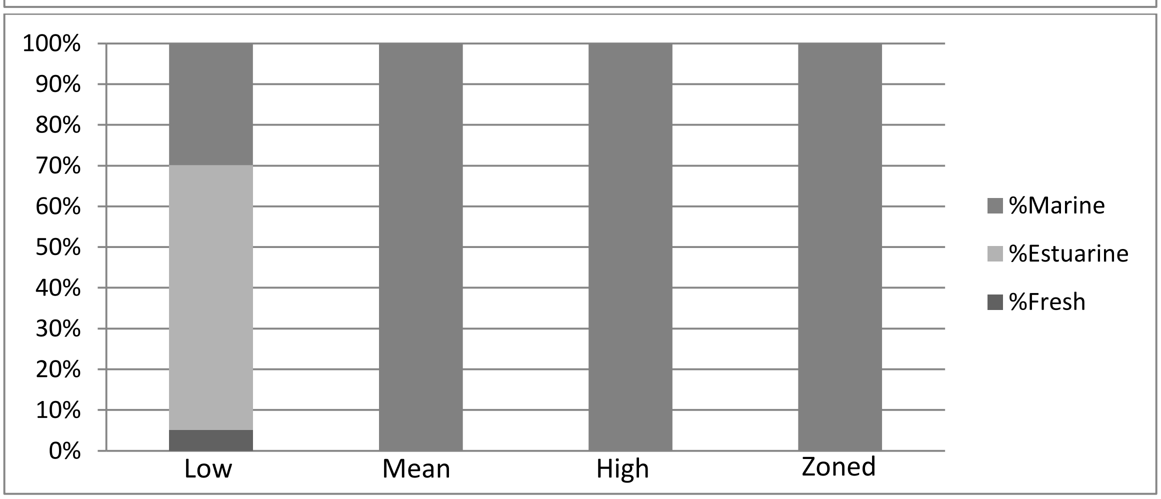
Figure AB.47. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 72. Figure 47.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.48. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 73. Figure 48.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

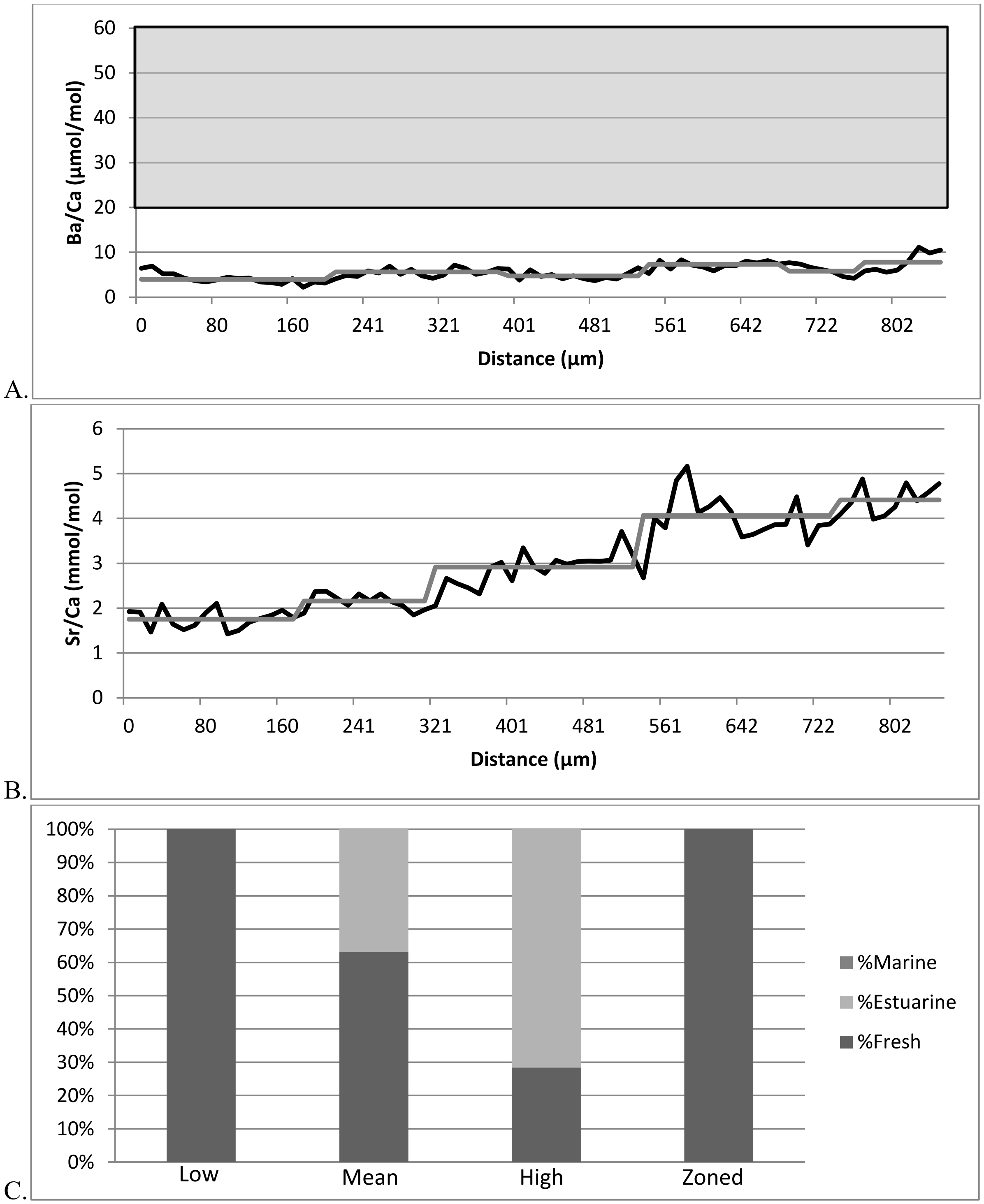
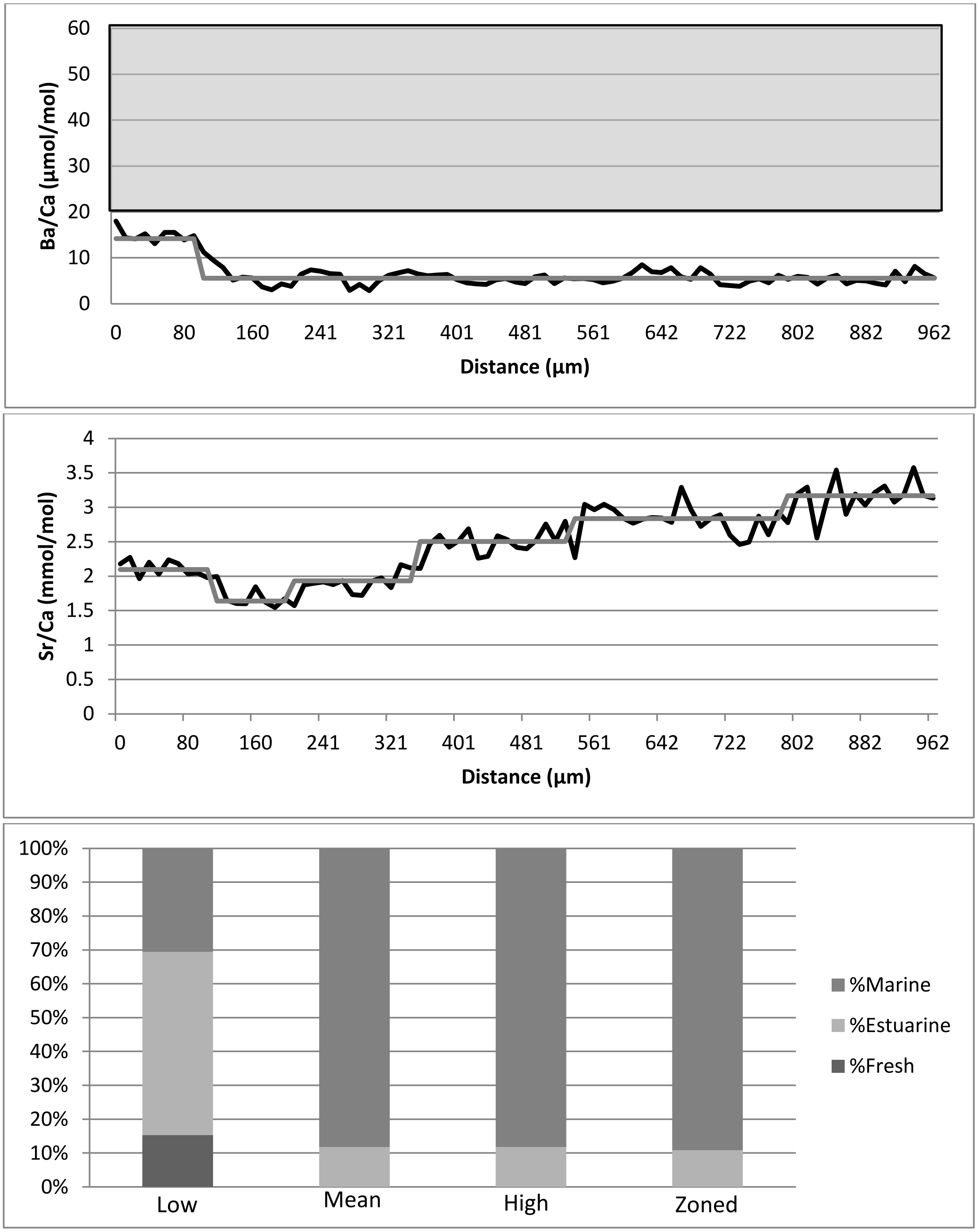
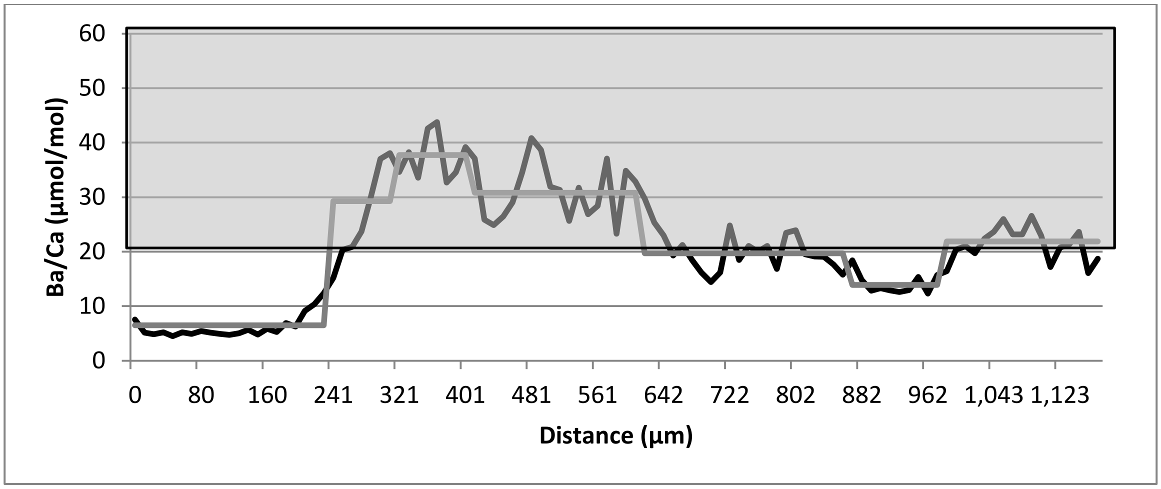


Figure AB.49. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 74. Figure 49.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

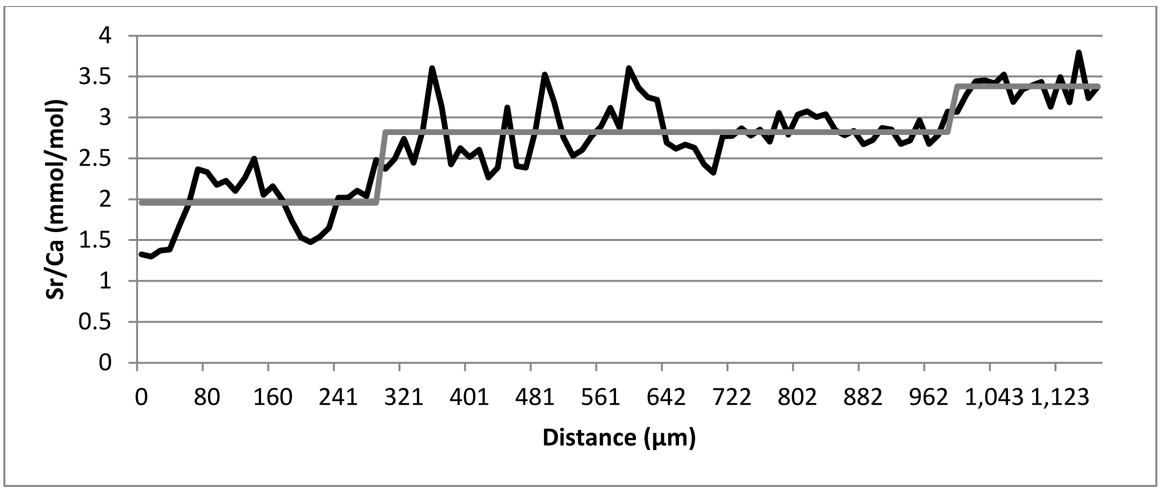


A.
B.
C.

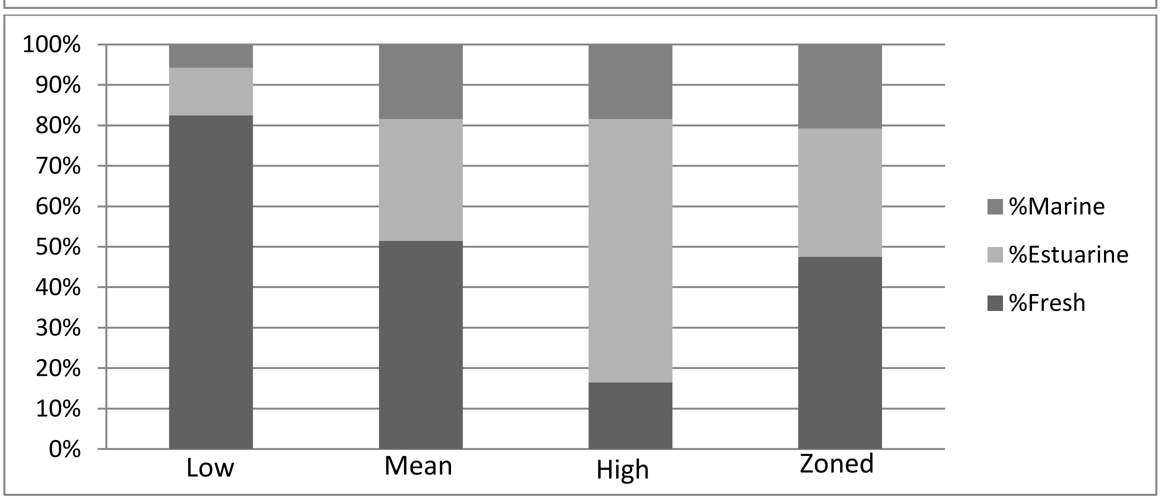
Figure AB.50. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 75. Figure 50.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

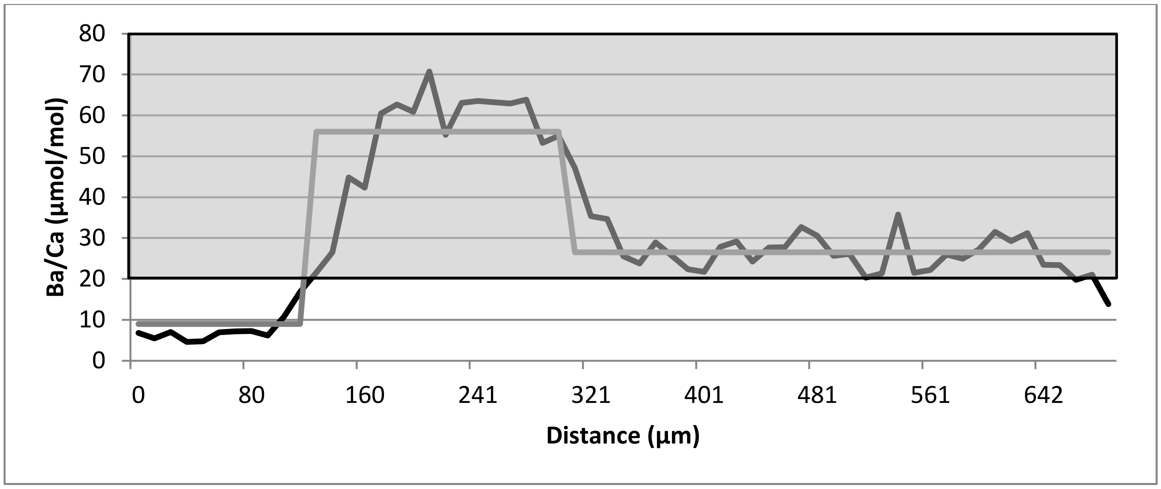


B.

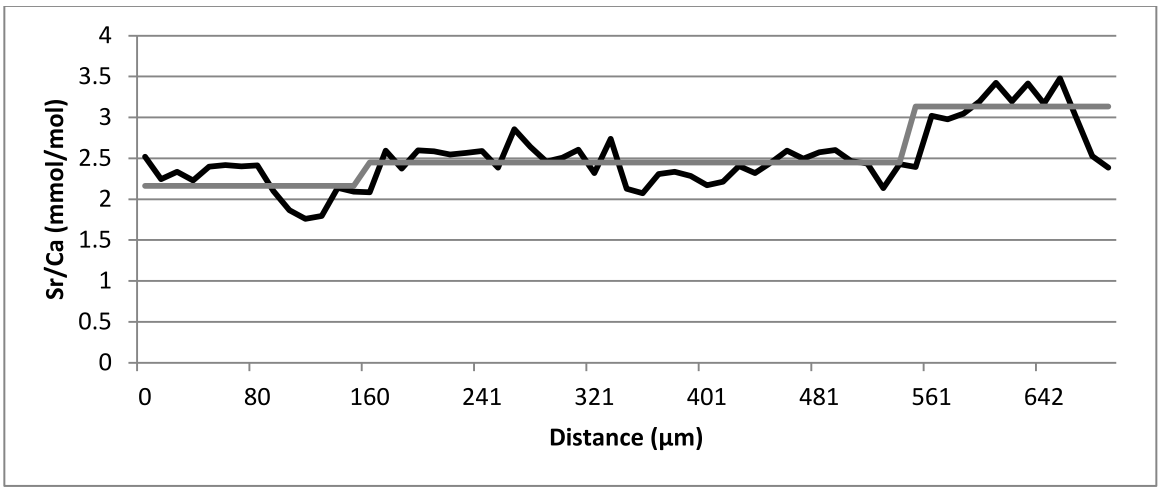


C.

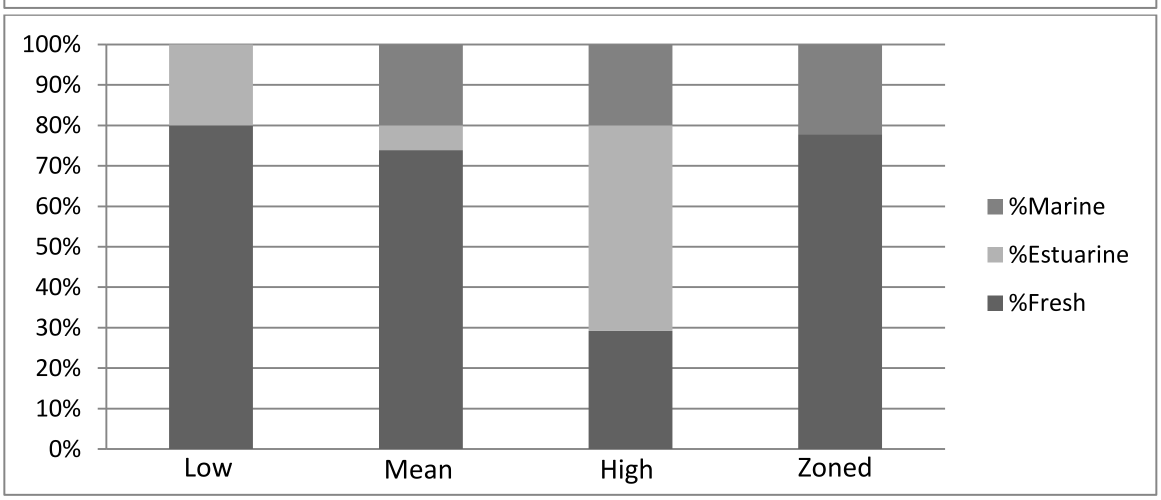
Figure AB.51. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 76. Figure 51.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

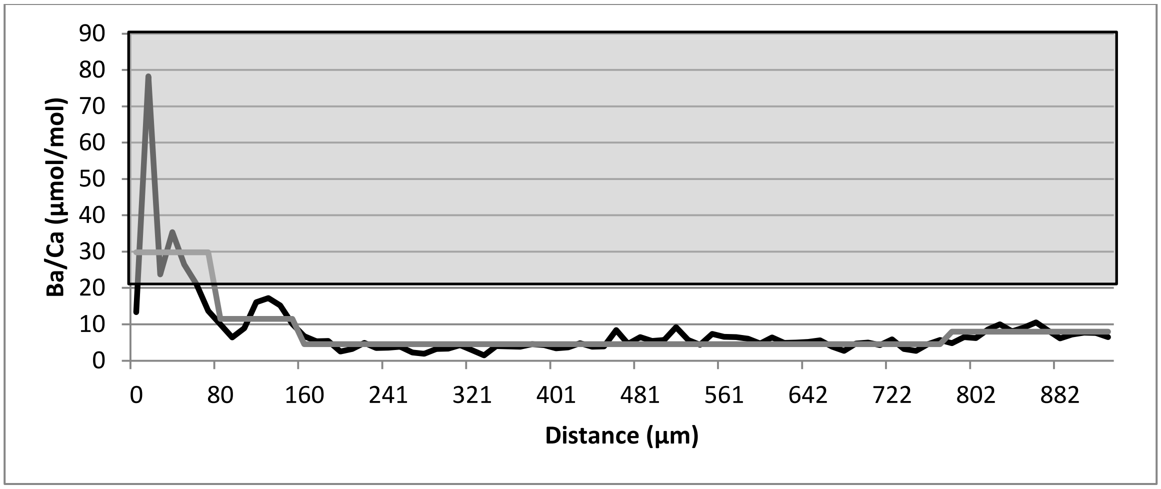


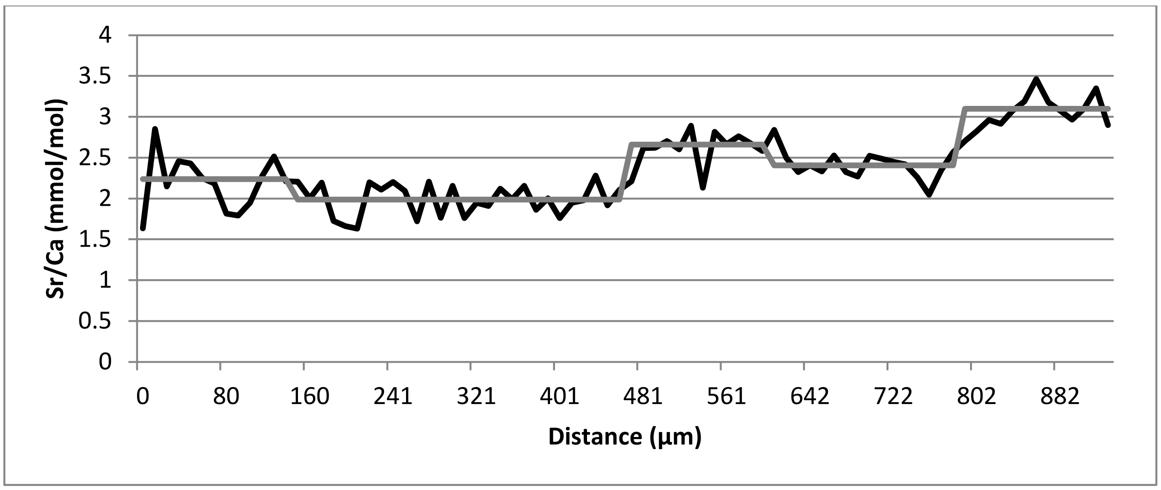
B.



C.

Figure AB.52. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 77. Figure 52.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


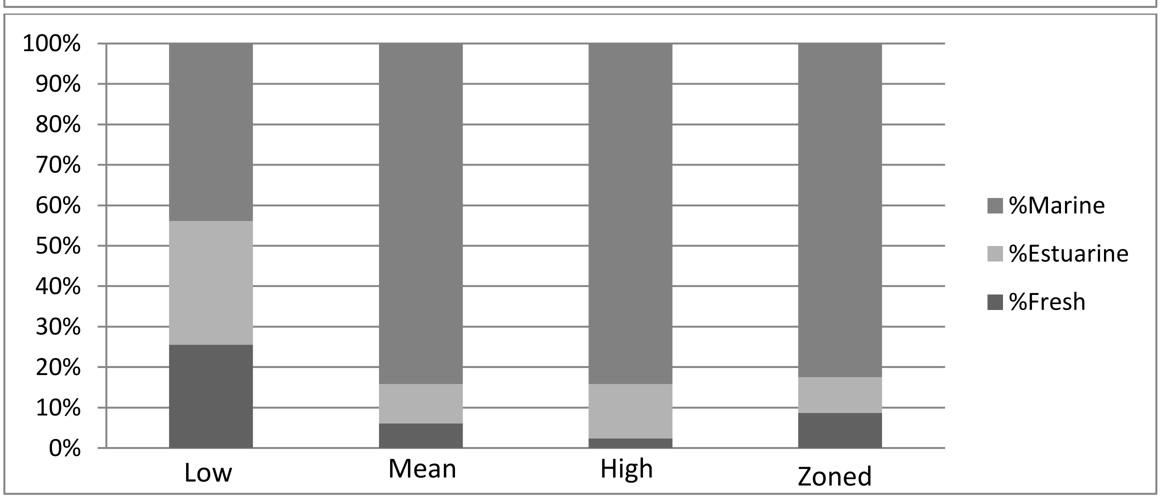
C.


Figure AB.53. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 78. Figure 53.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

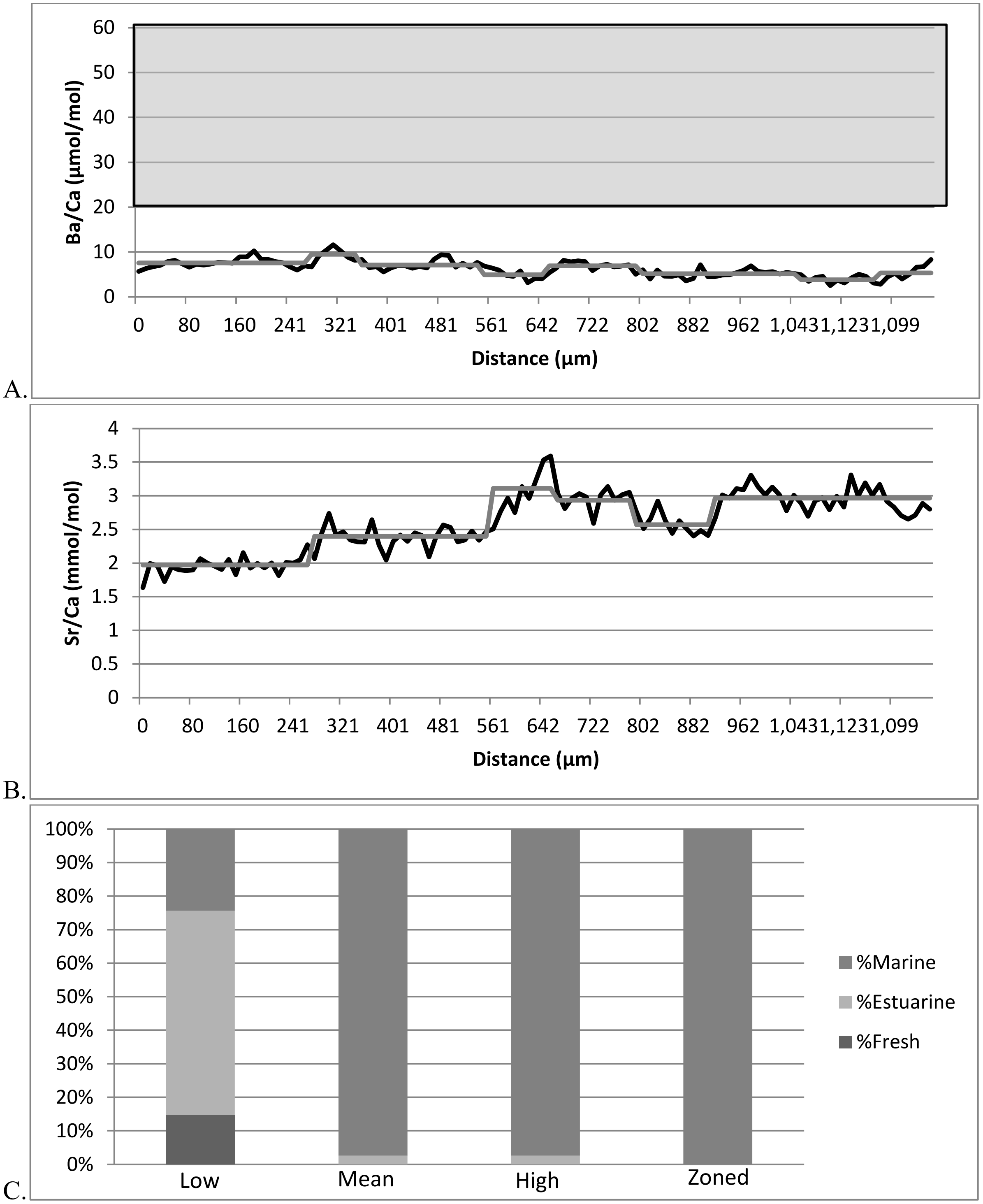
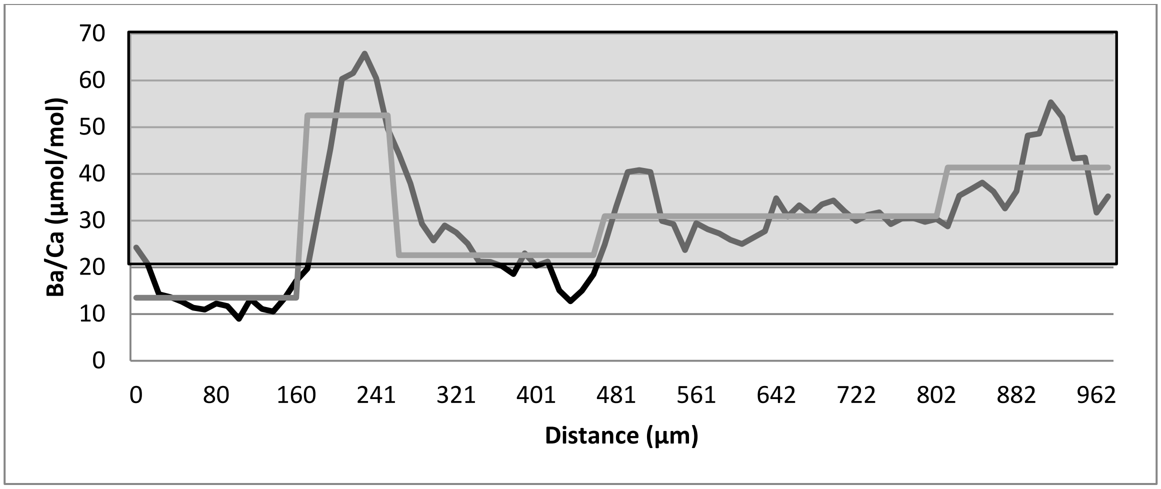
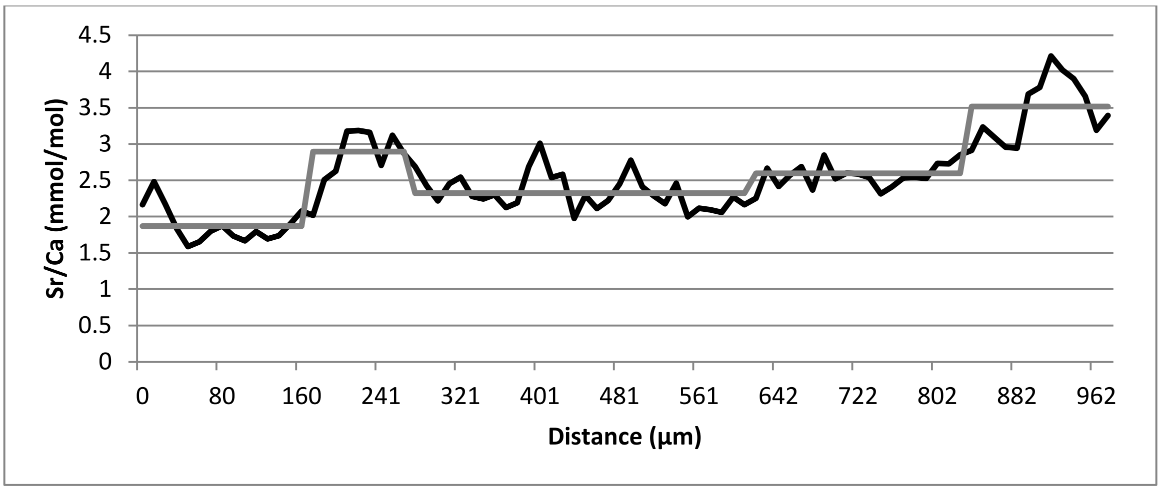


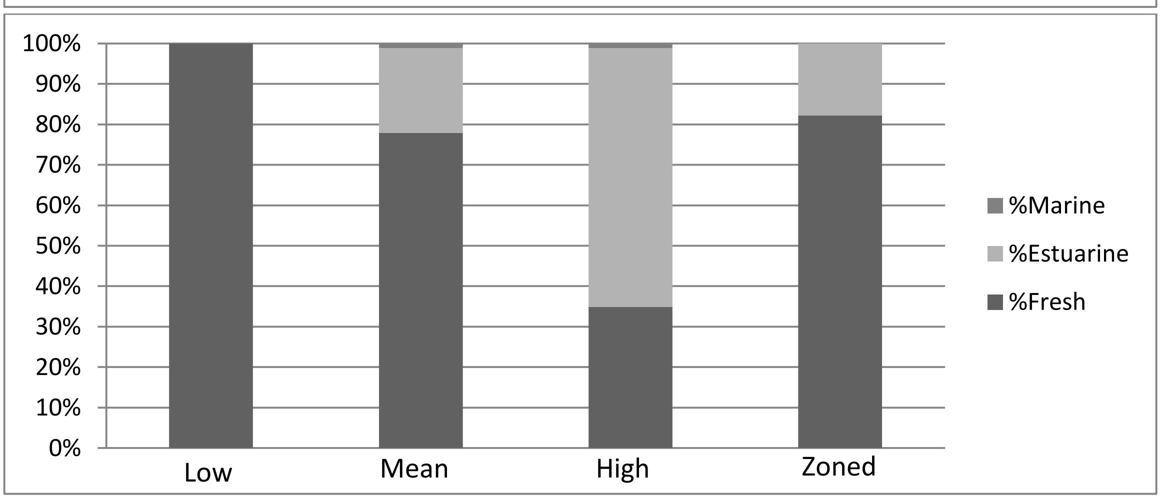
Figure AB.54. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 79. Figure 54.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

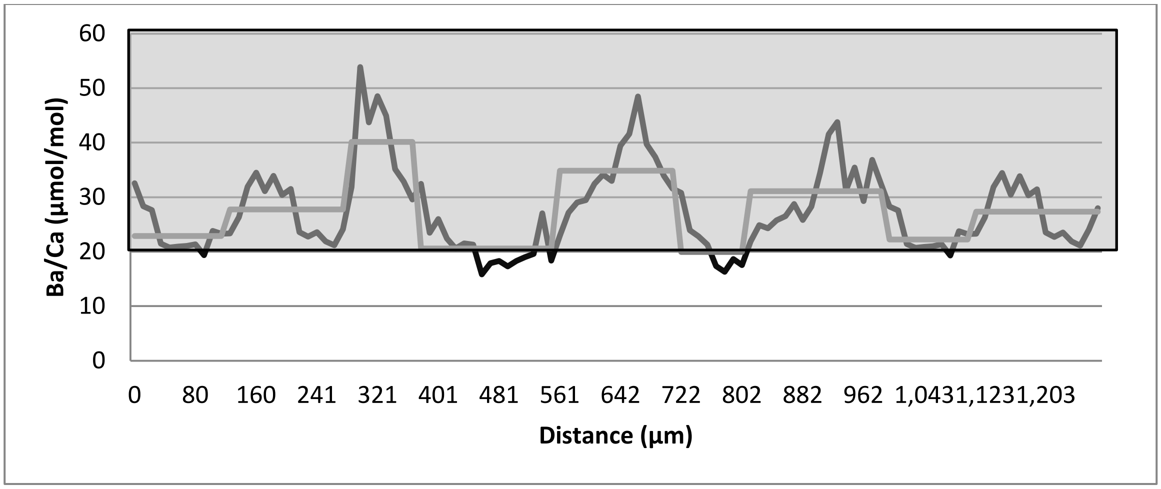


B.

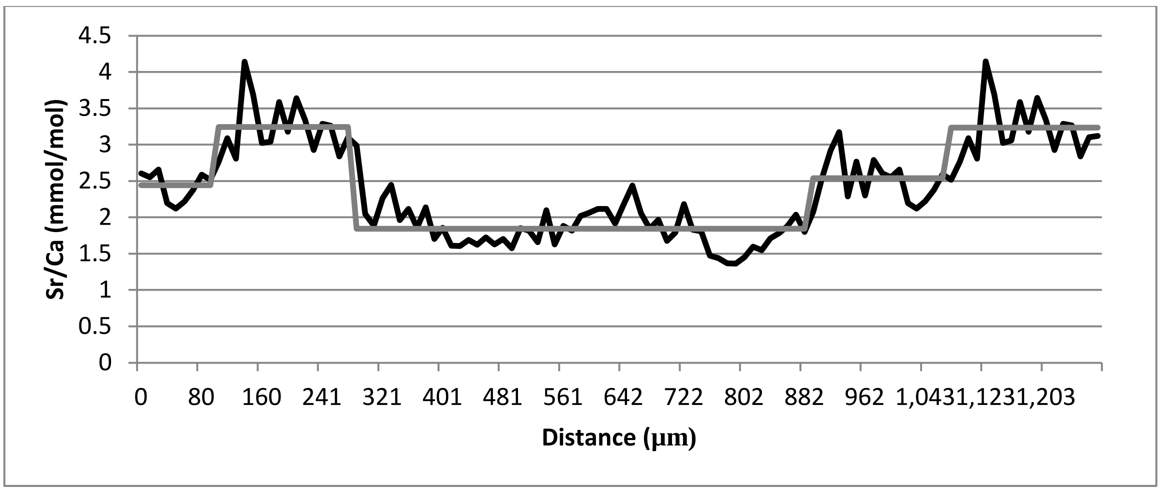


C.

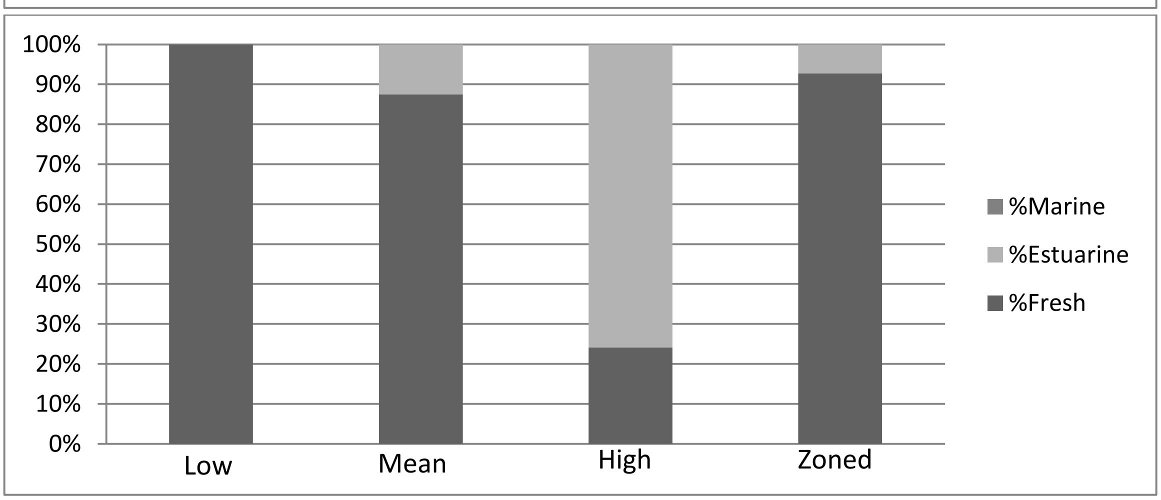
Figure AB.55. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 82. Figure 55.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

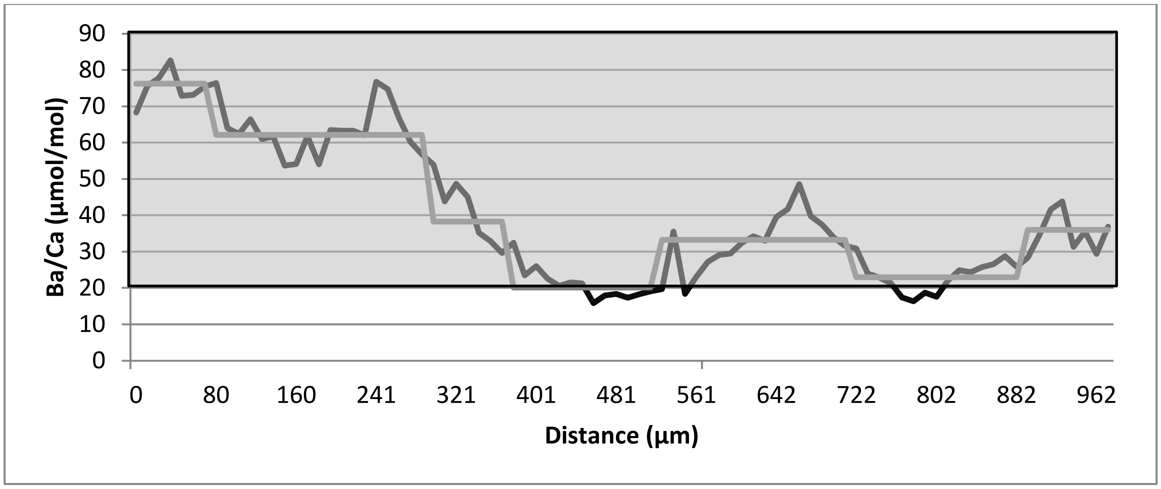


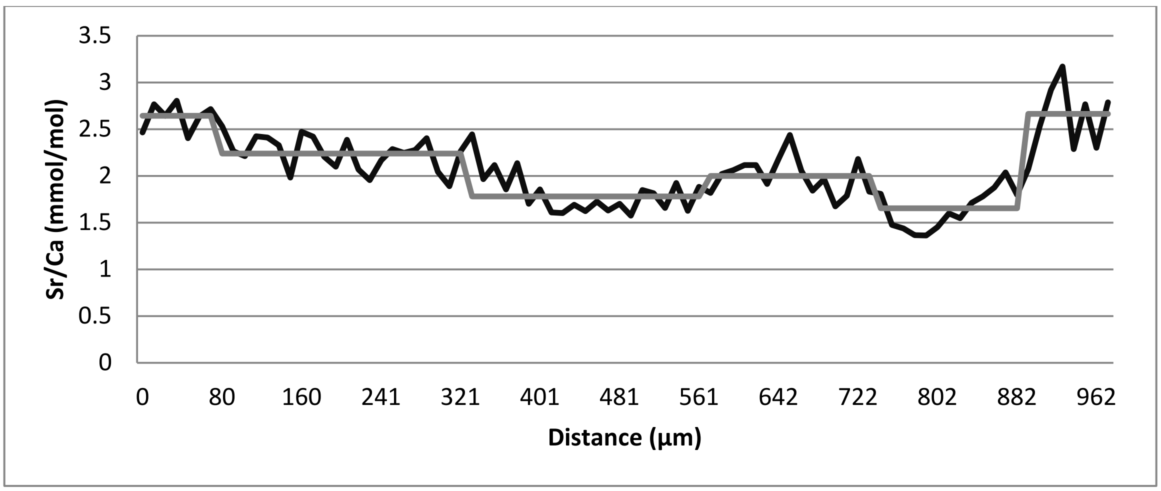
B.



C.

Figure AB.56. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 83. Figure 56.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


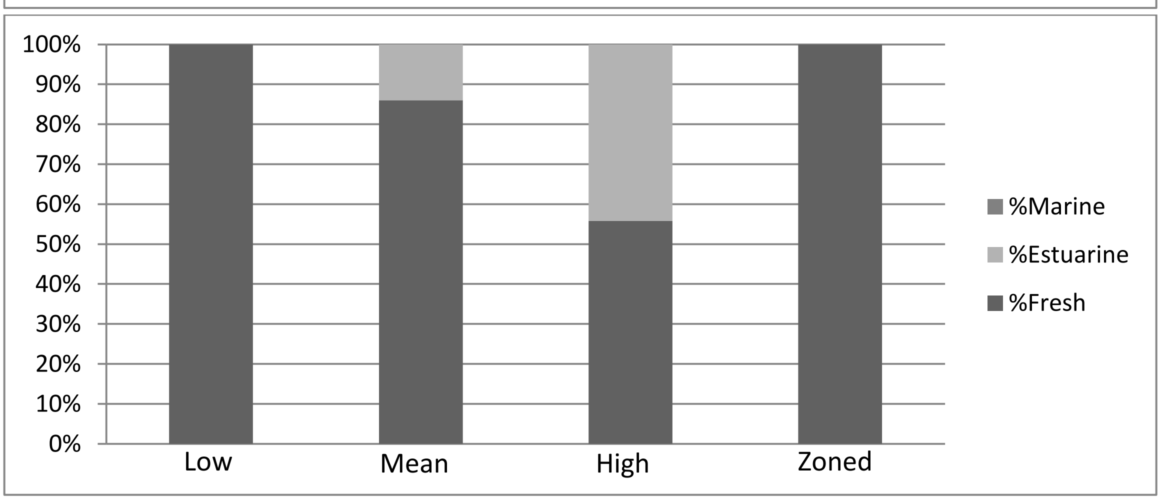
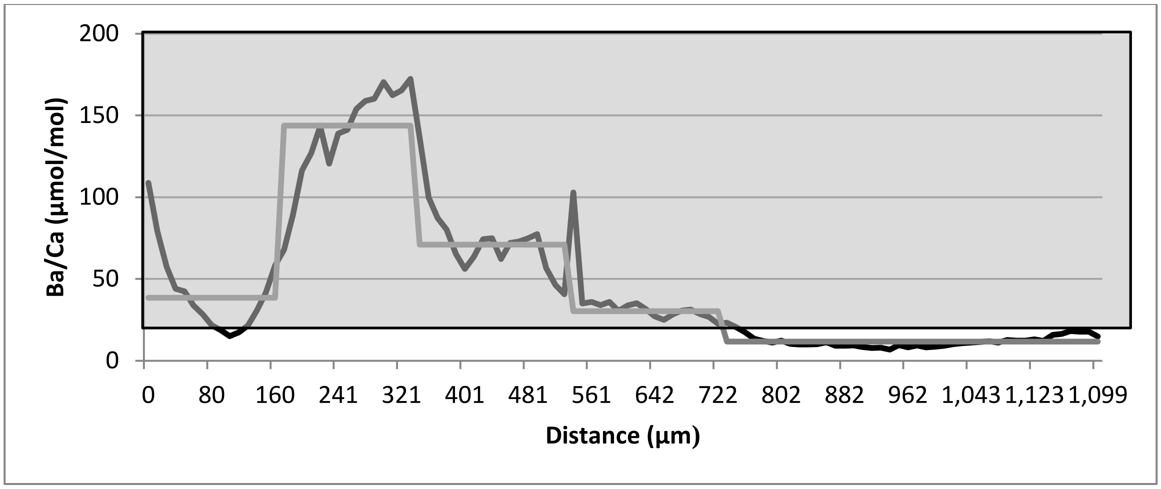
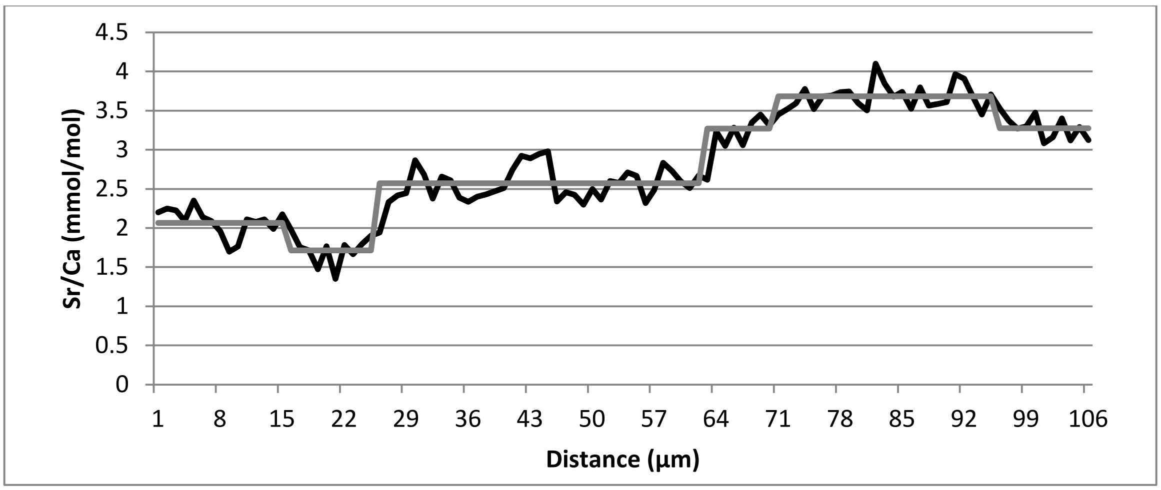
C.


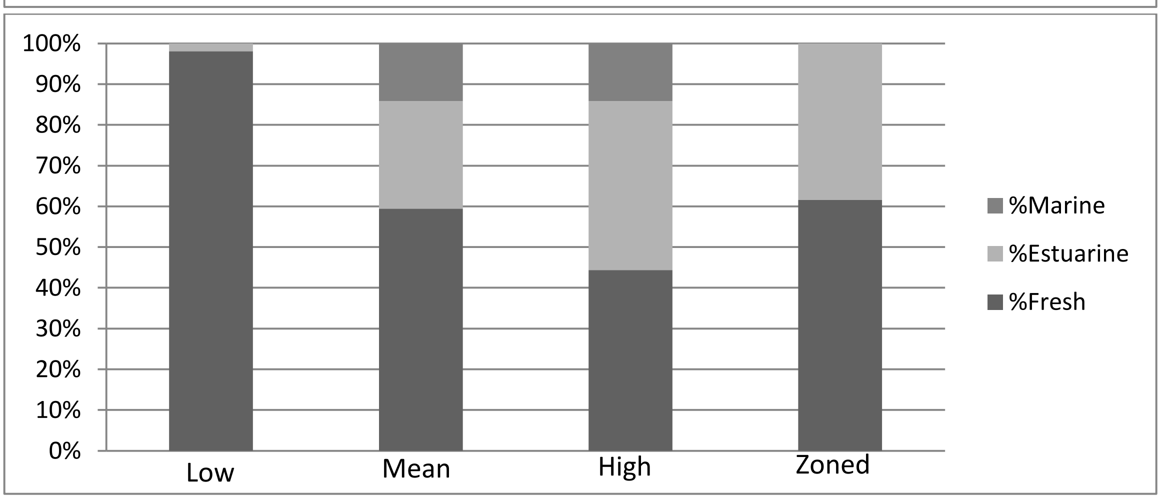
Figure AB.57. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 84. Figure 57.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

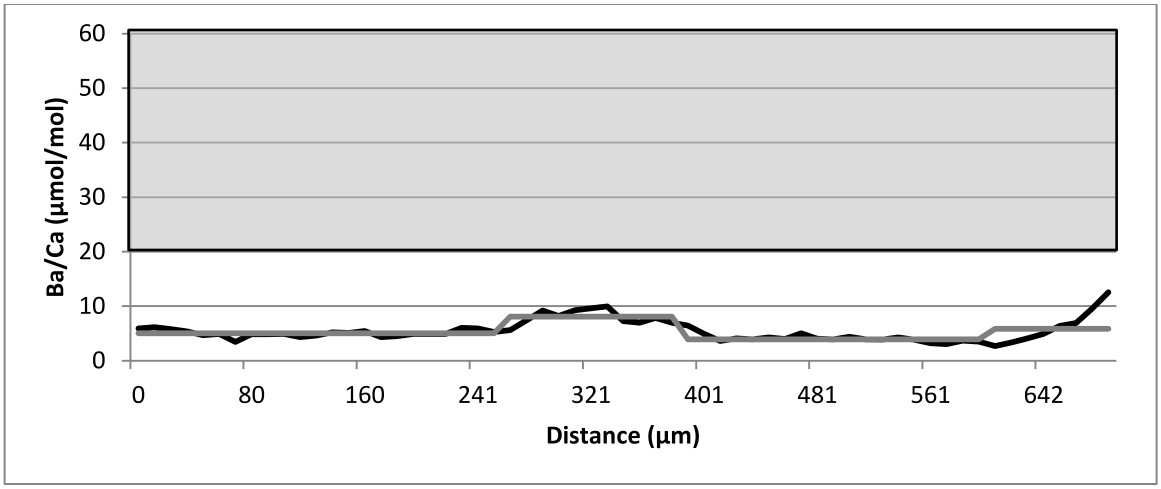


B.

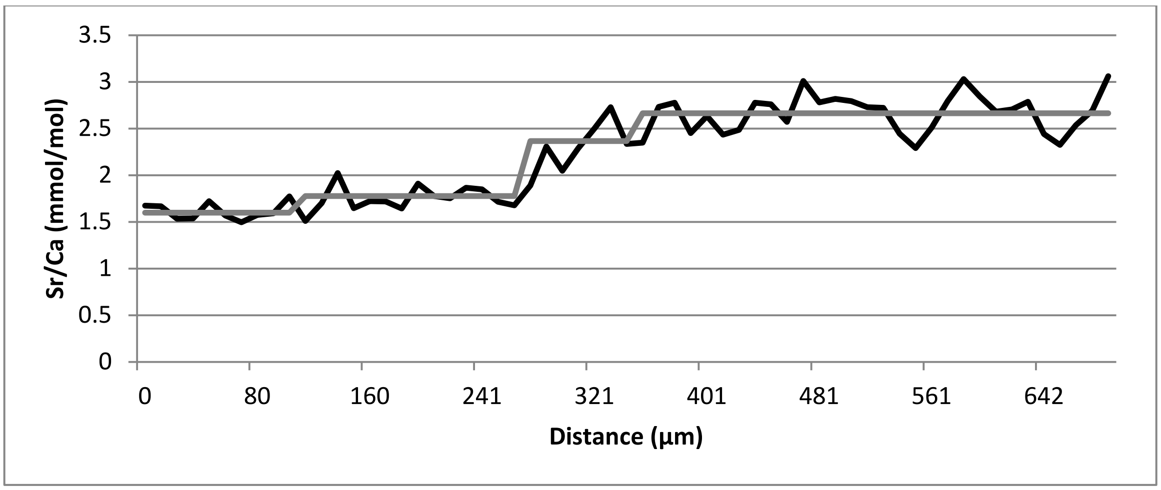


C.

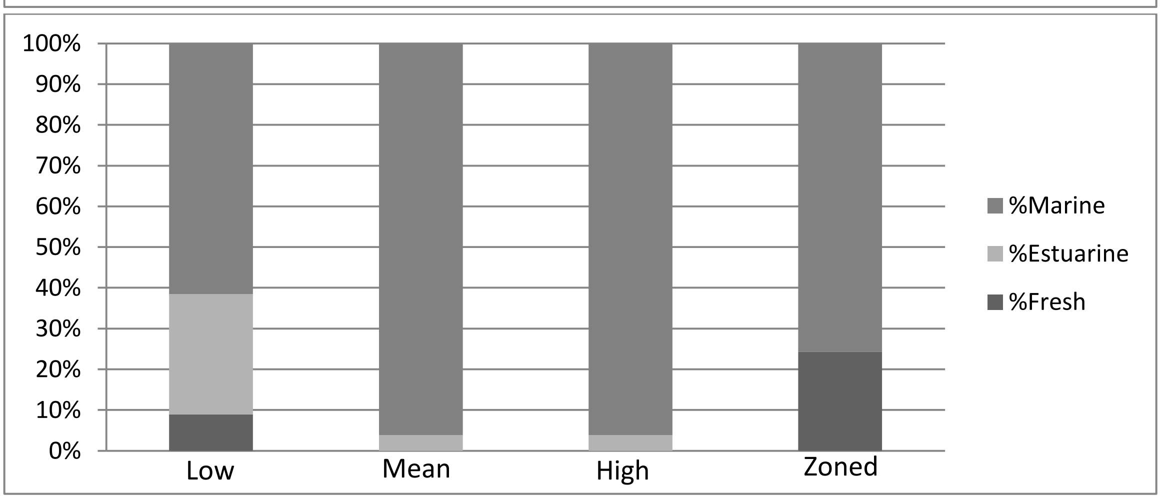
Figure AB.58. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 87. Figure 58.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

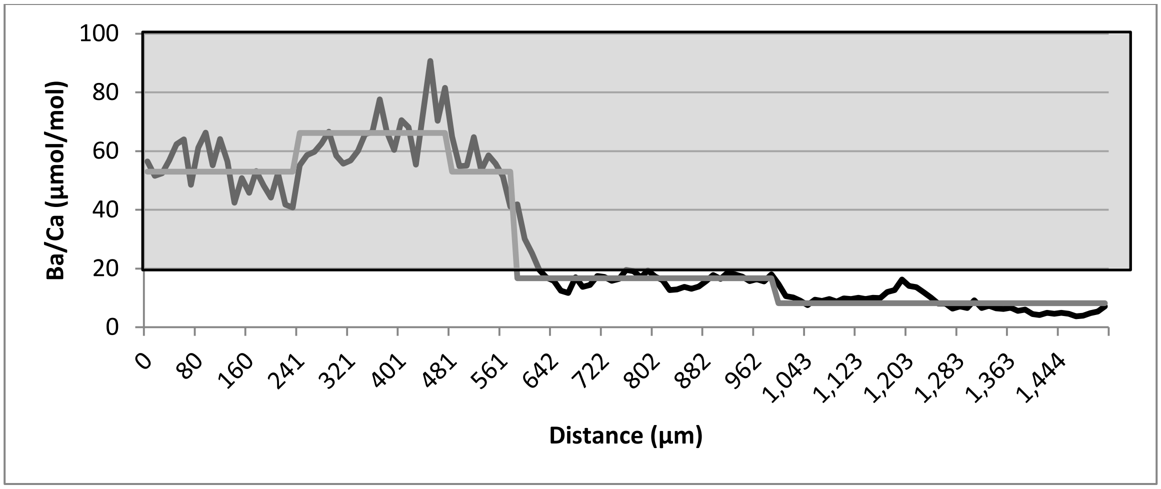


B.

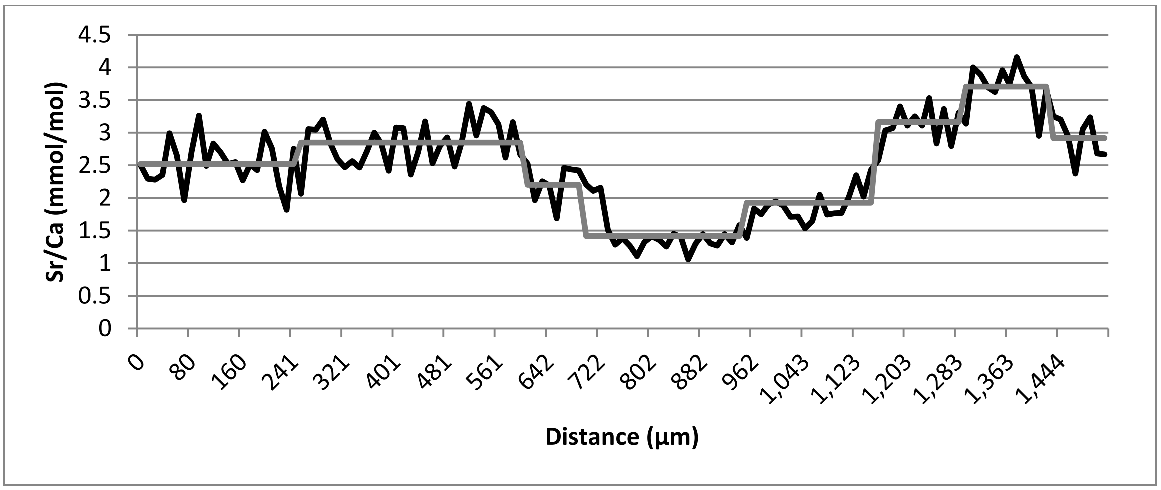


C.

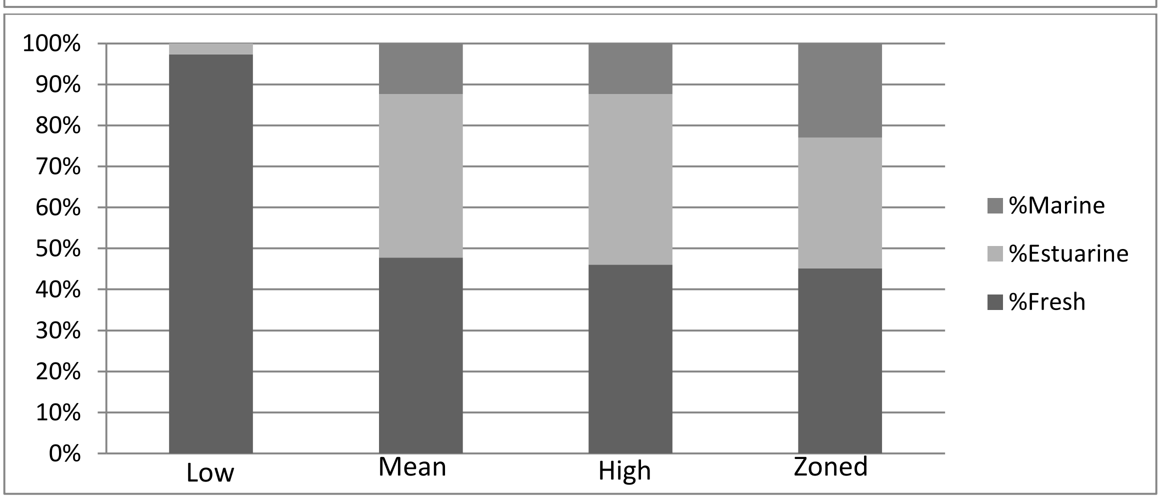
Figure AB.59. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 88. Figure 59.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

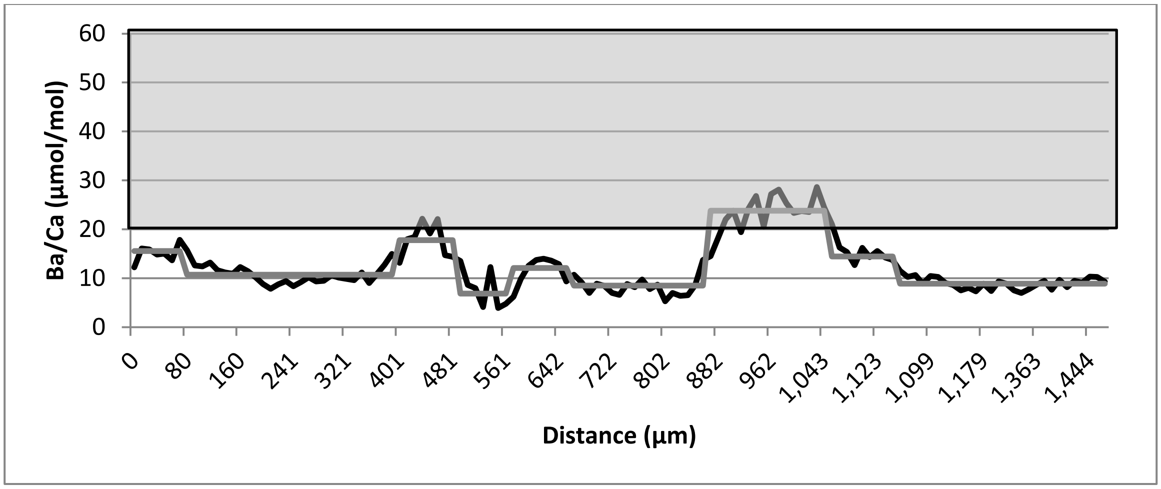


B.

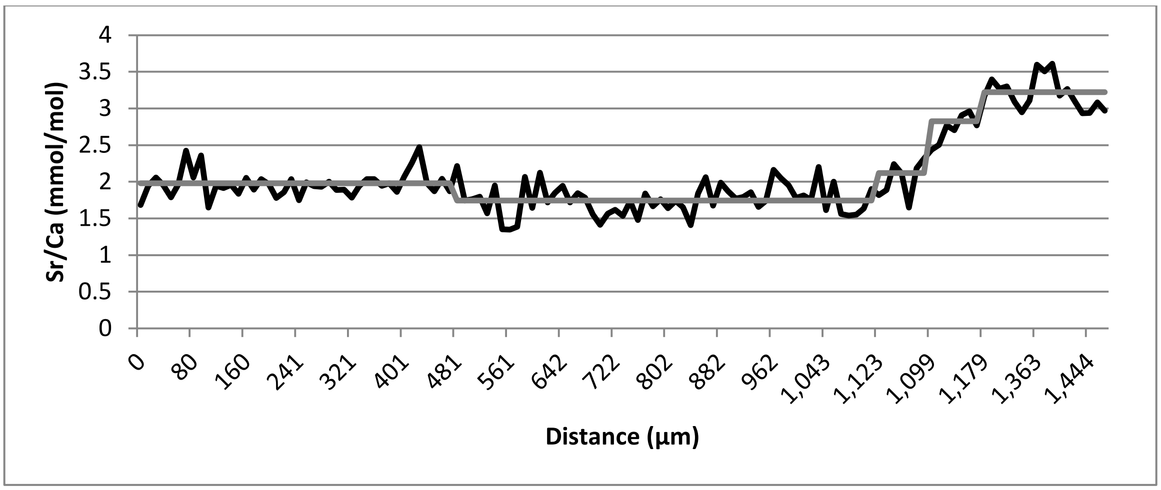


C.

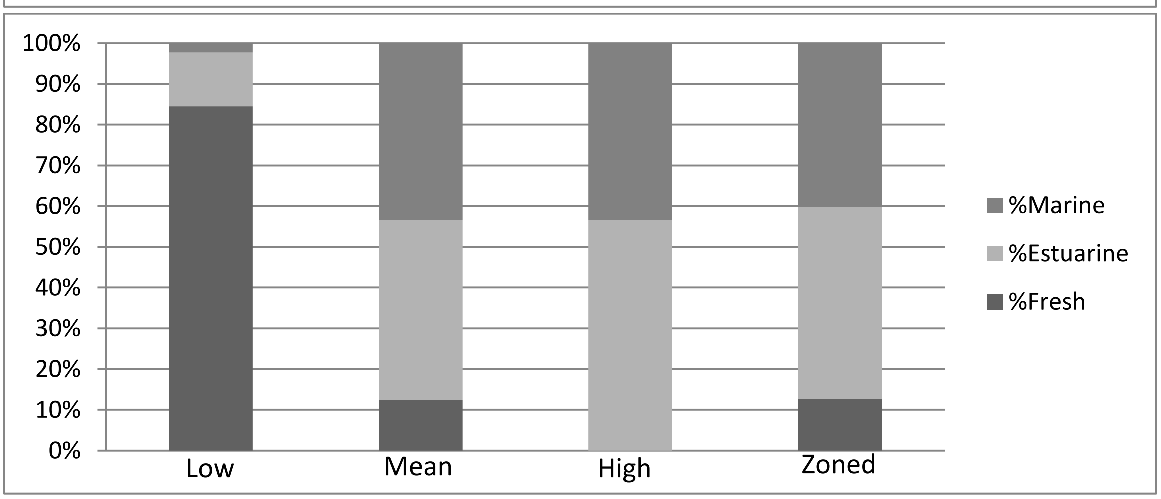
Figure AB.60. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 89. Figure 60.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

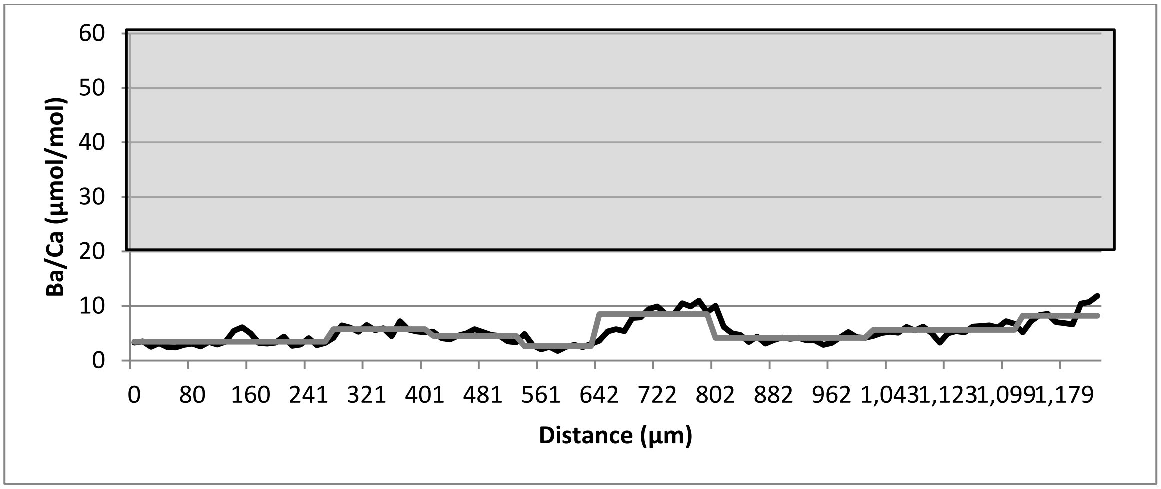


B.

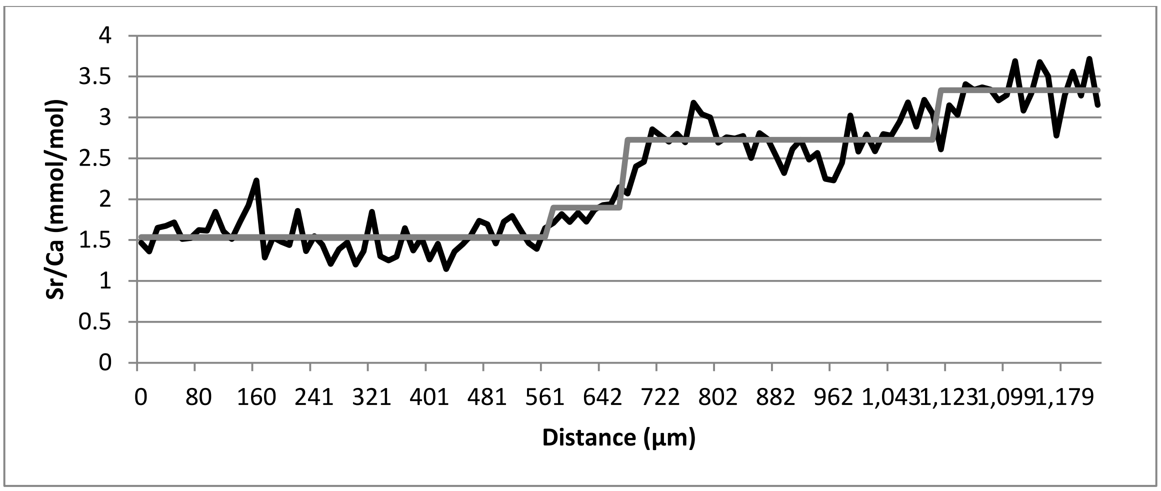


C.

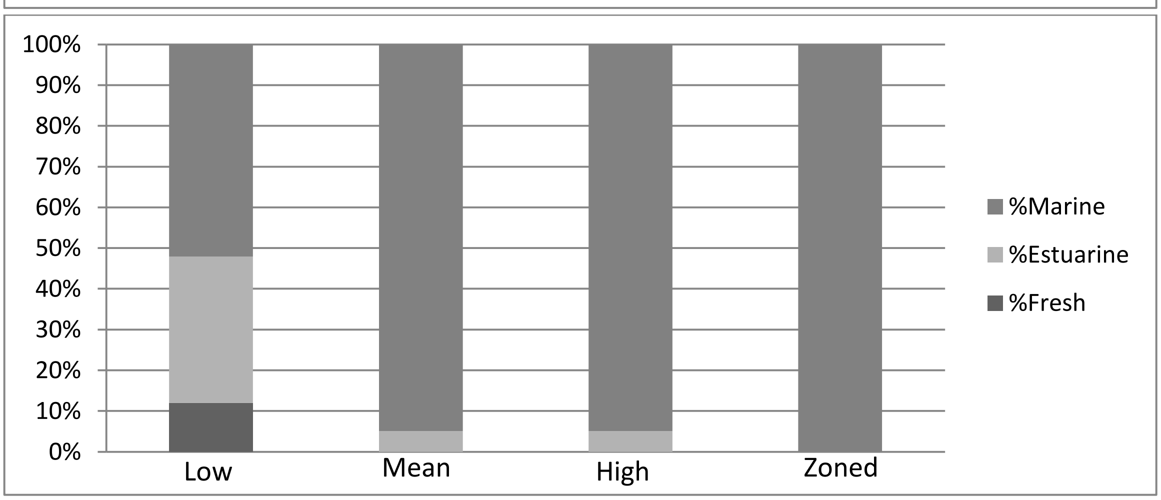
Figure AB.61. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 90. Figure 61.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

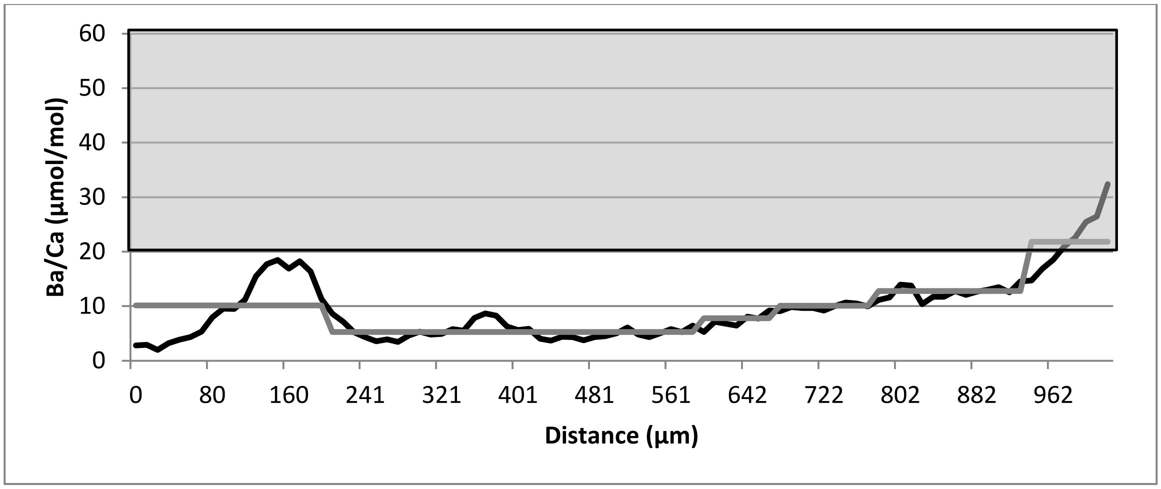


B.

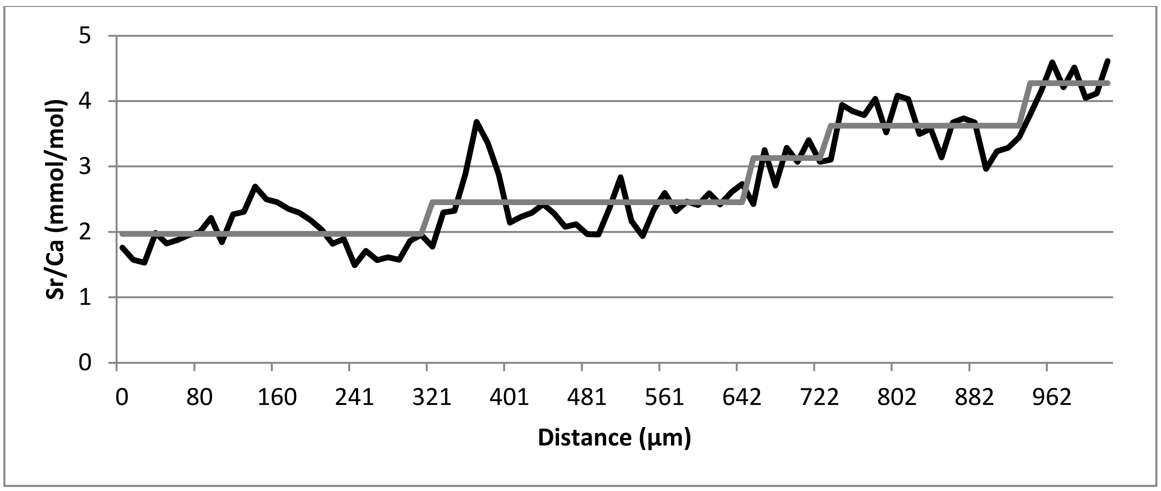


C.

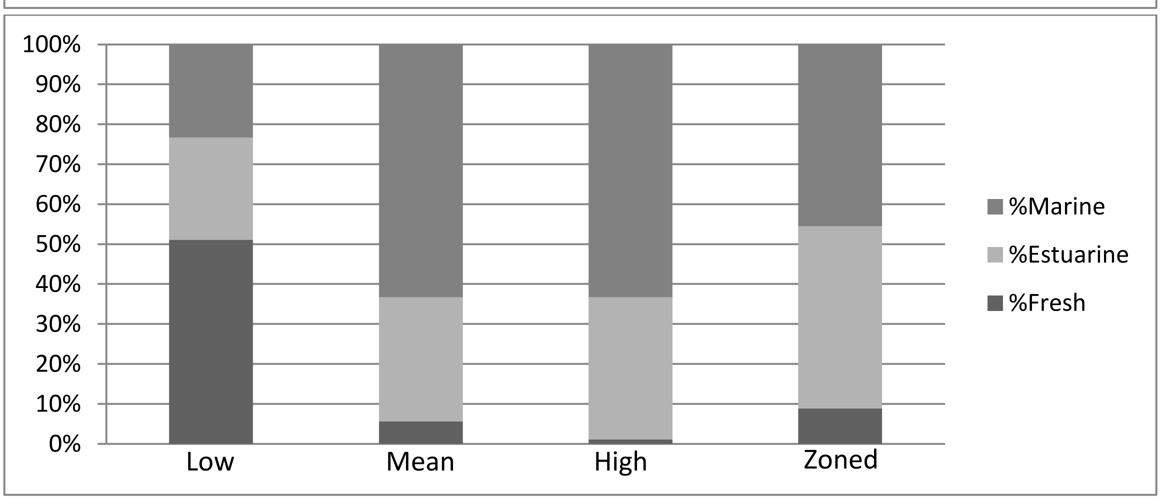
Figure AB.62. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 91. Figure 62.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

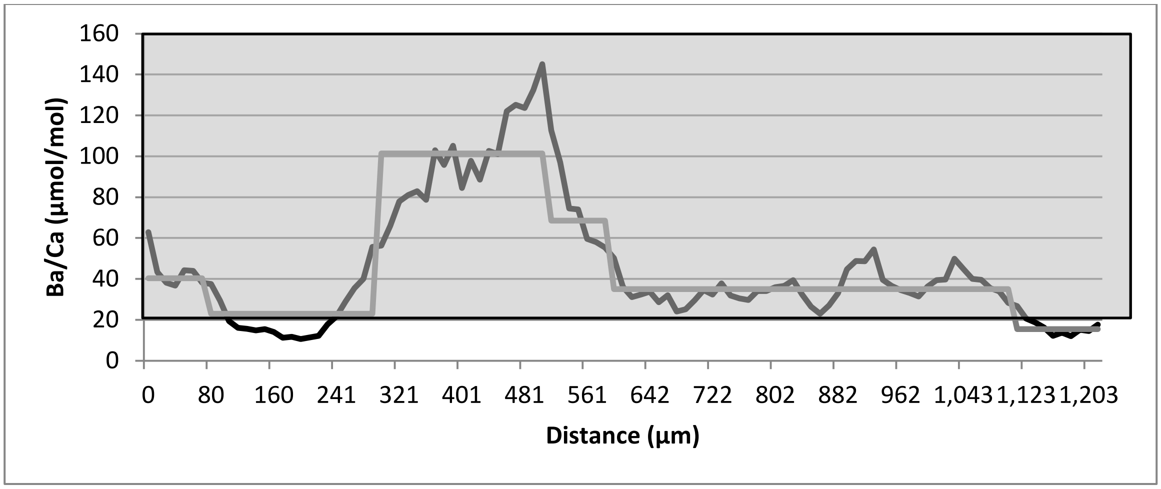


B.

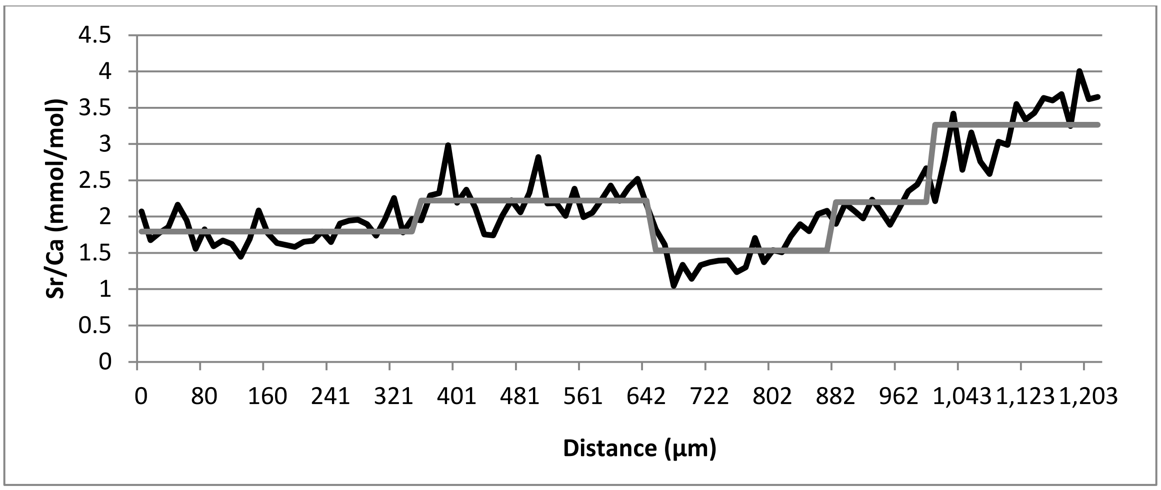


C.

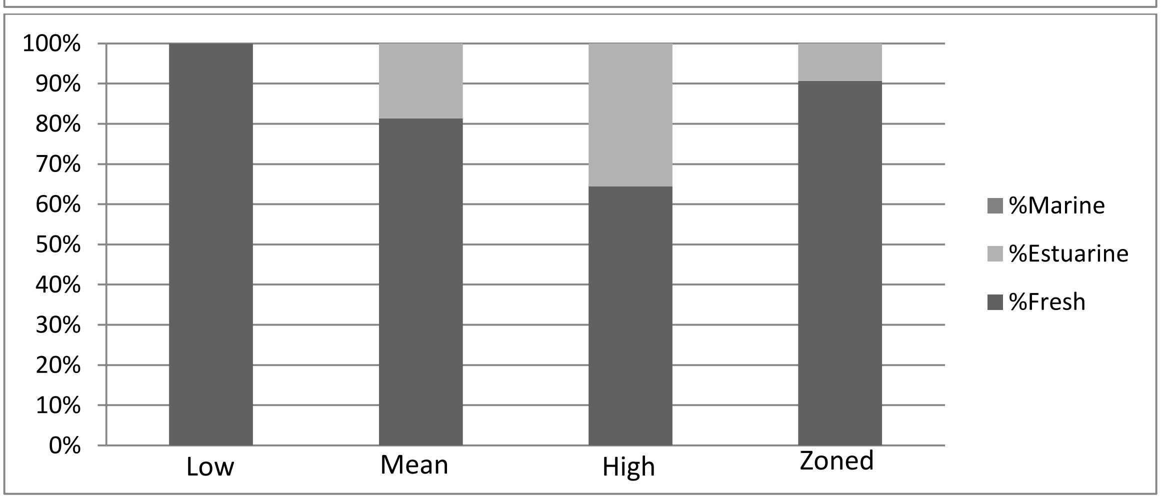
Figure AB.63. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 93. Figure 63.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.64. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 94. Figure 64.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

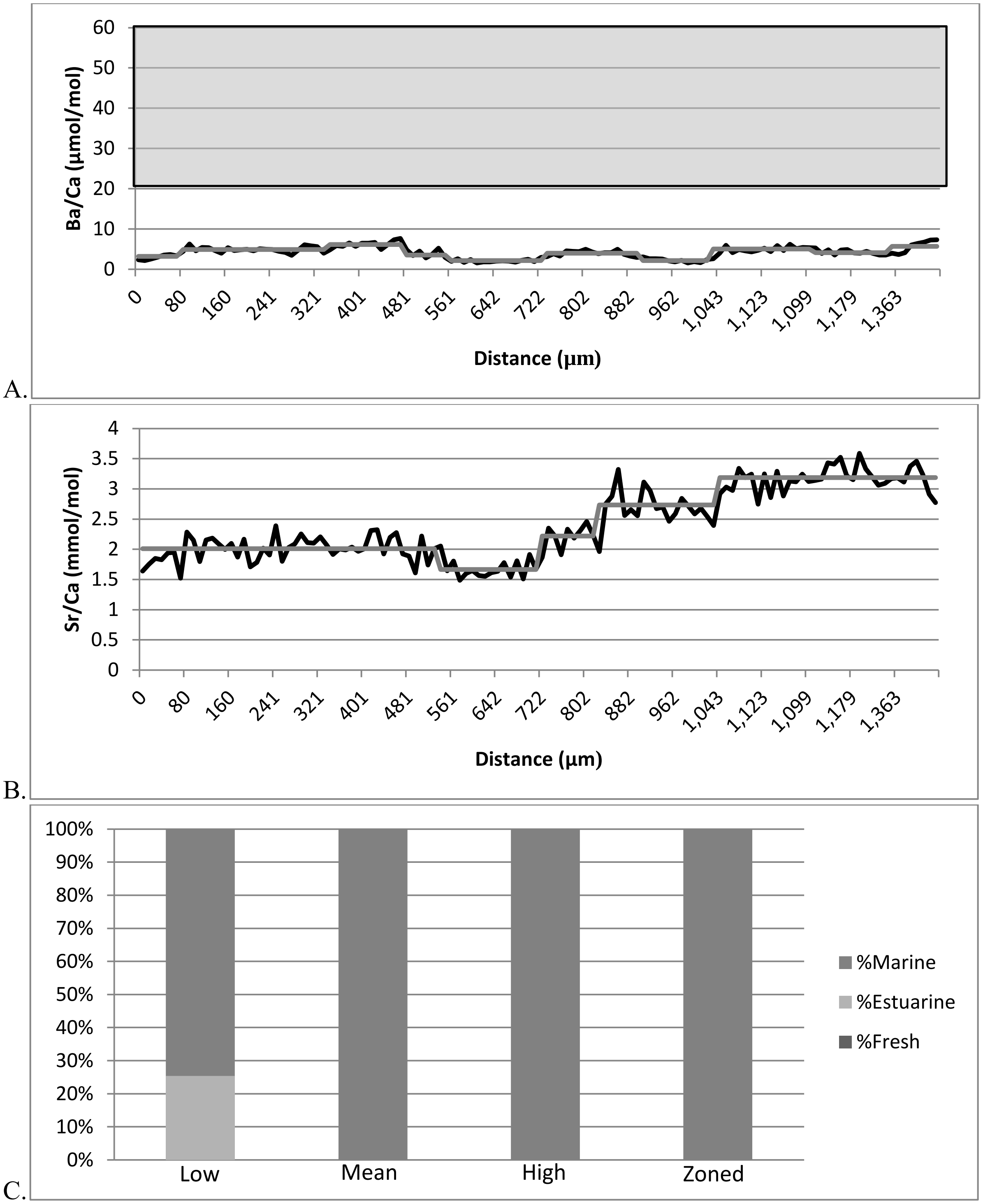


Figure AB.65. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 95. Figure 65.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

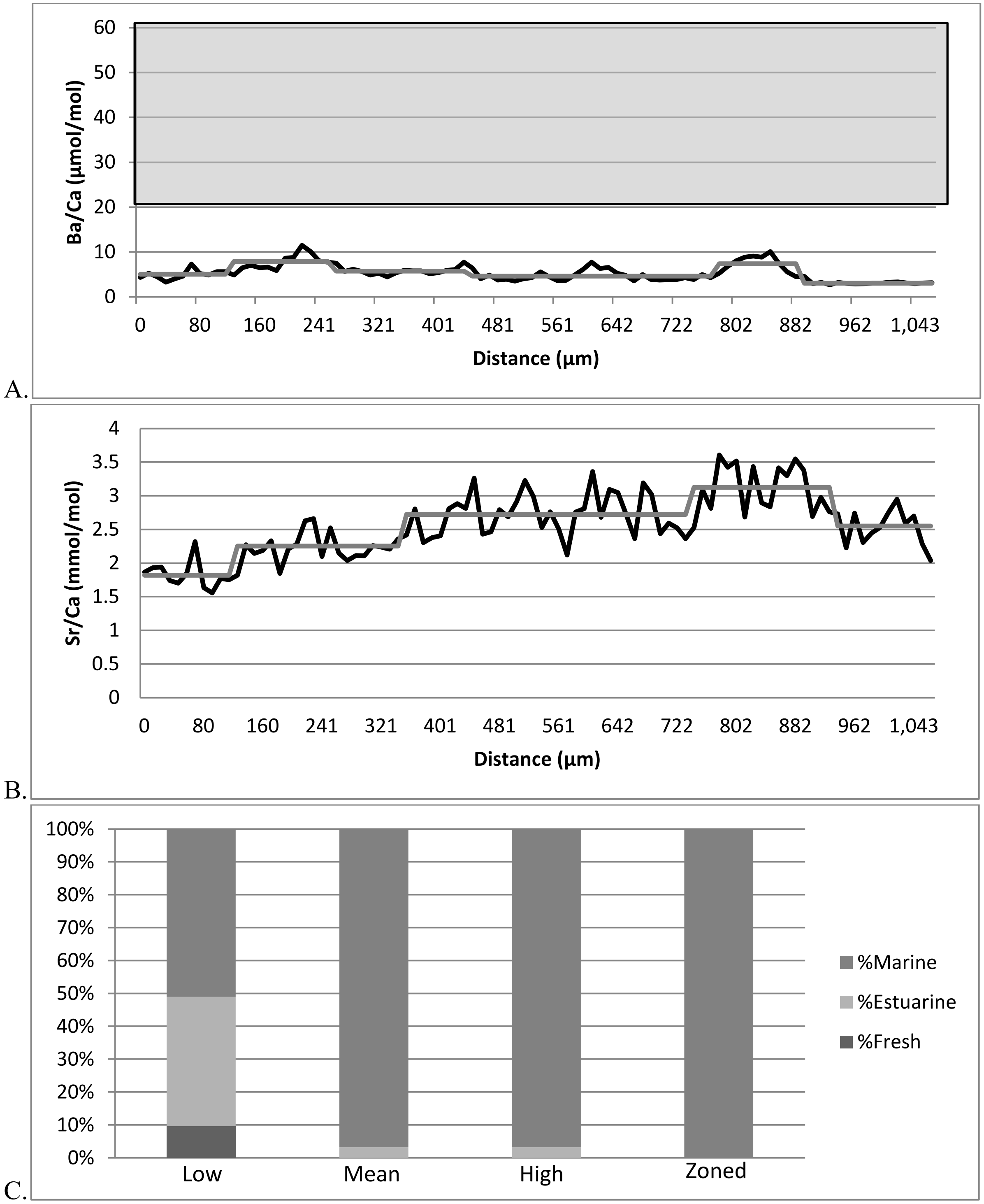
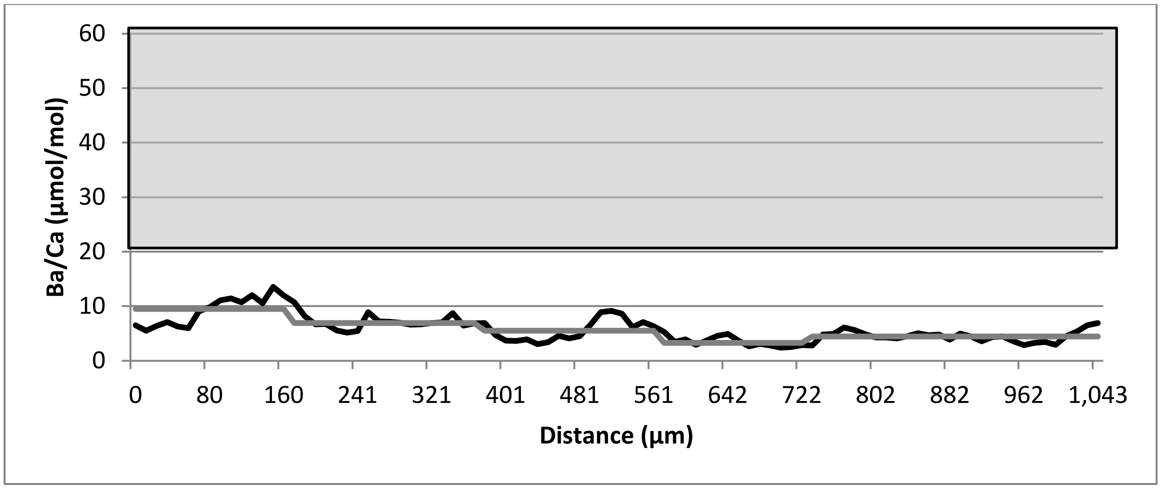
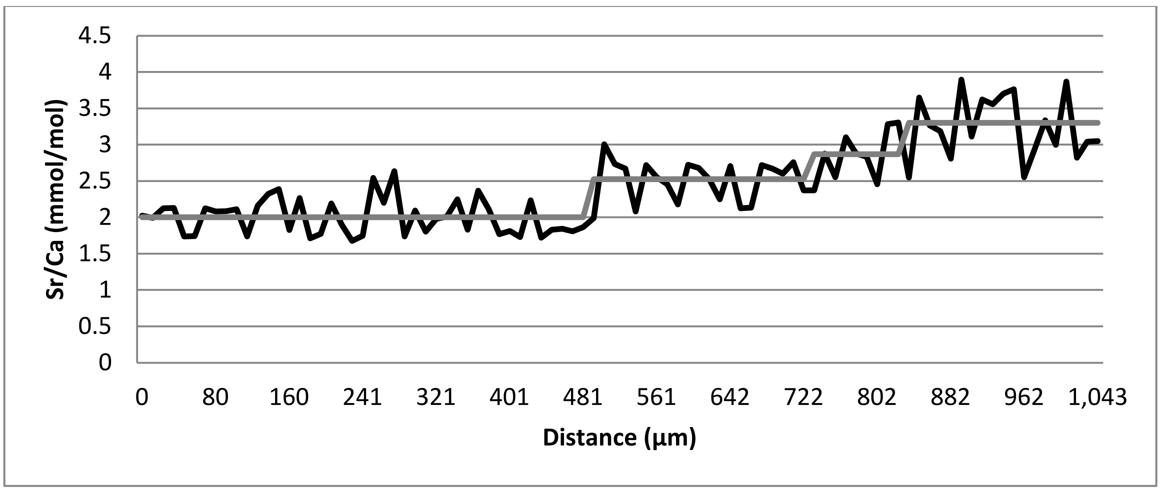


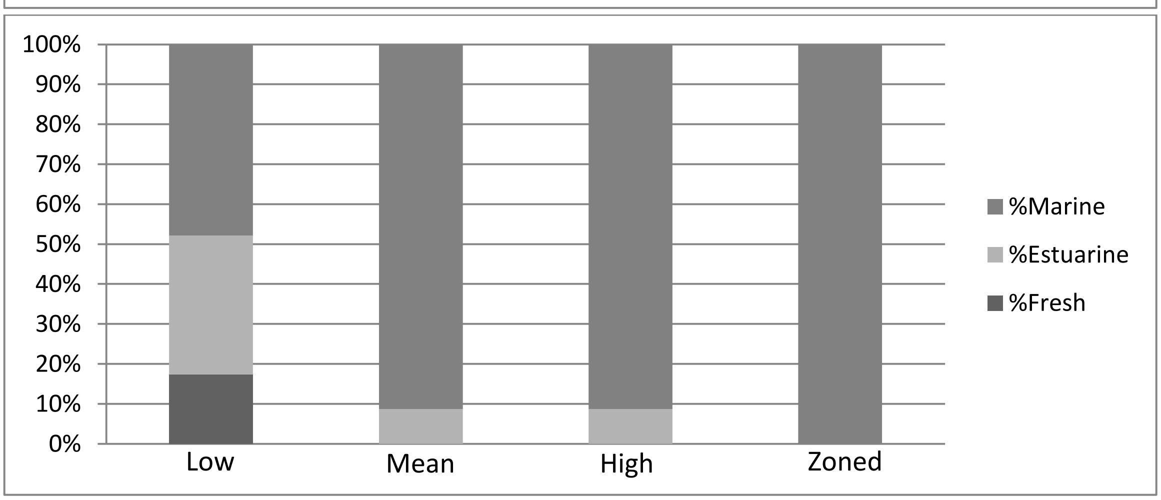
Figure AB.66. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 97. Figure 66.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

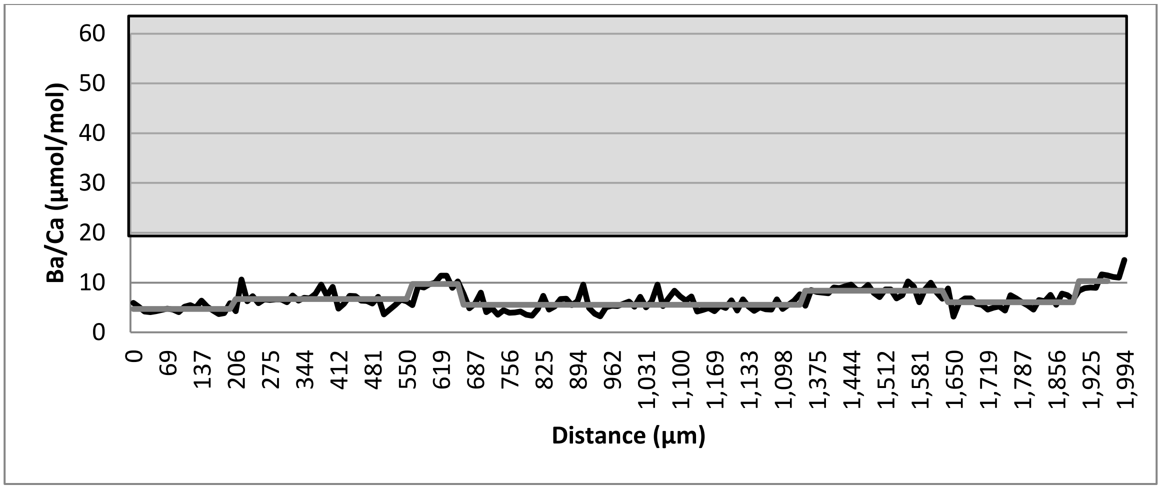


B.

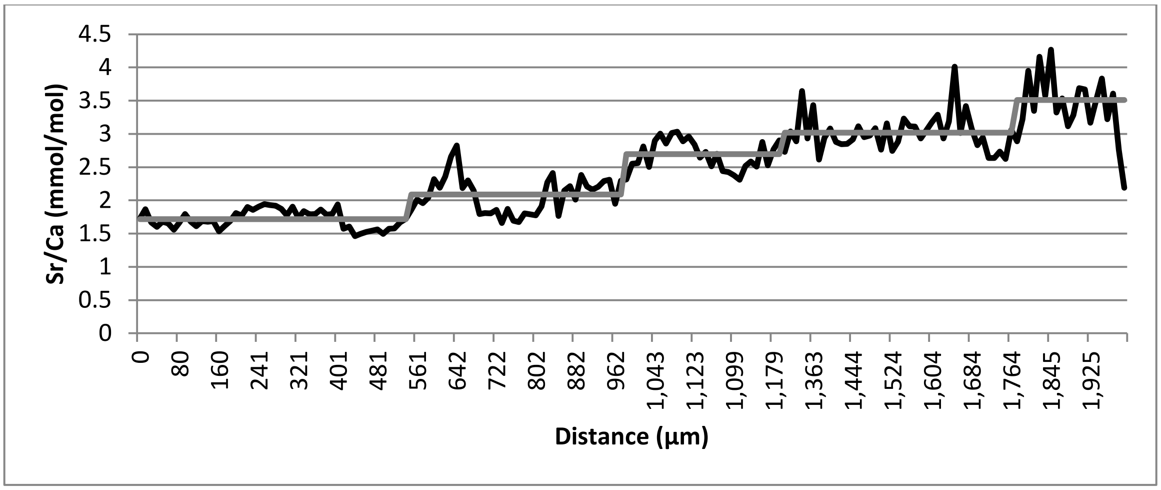


C.

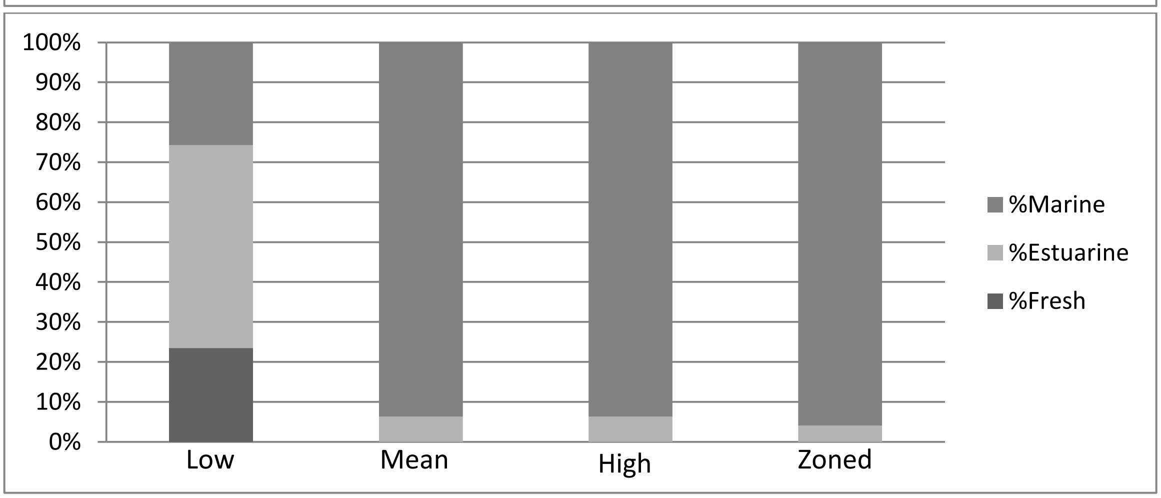
Figure AB.67. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 98. Figure 67.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

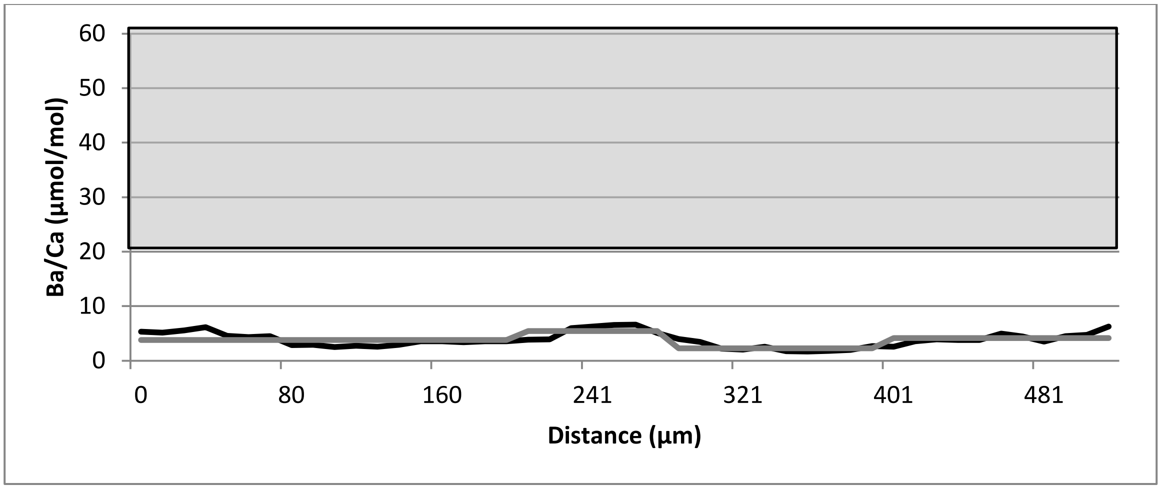


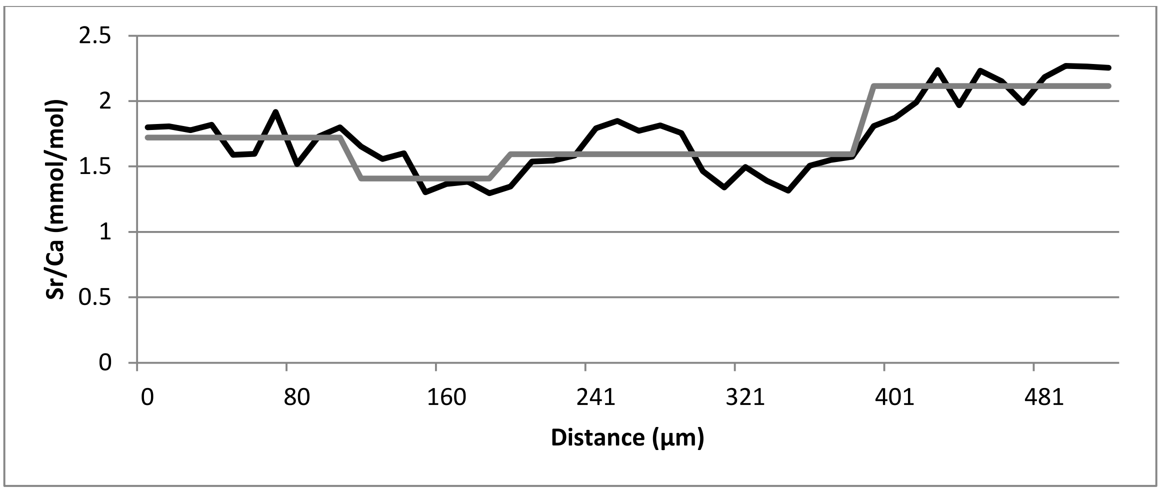
B.

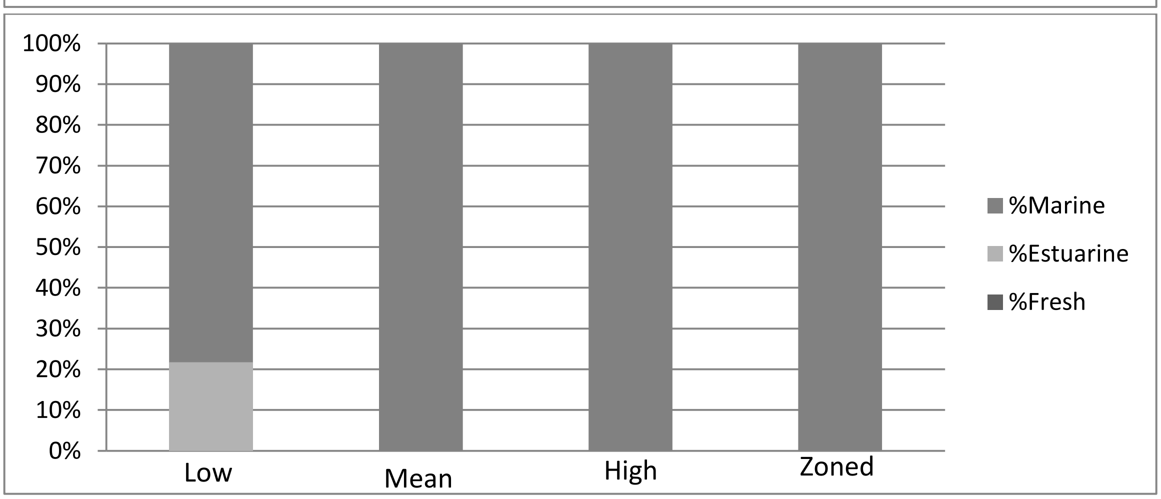


C.

Figure AB.68. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 99. Figure 68.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

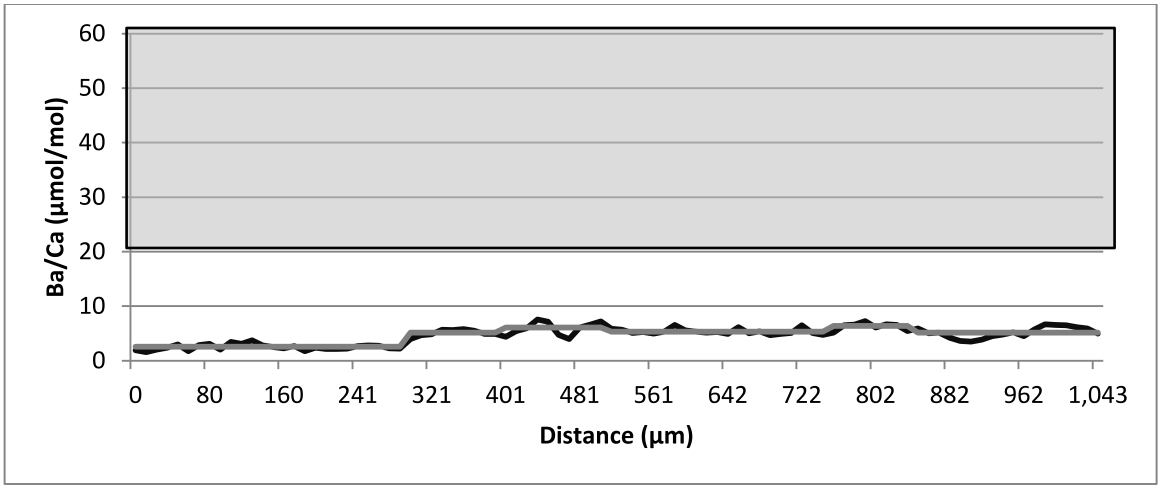


A.


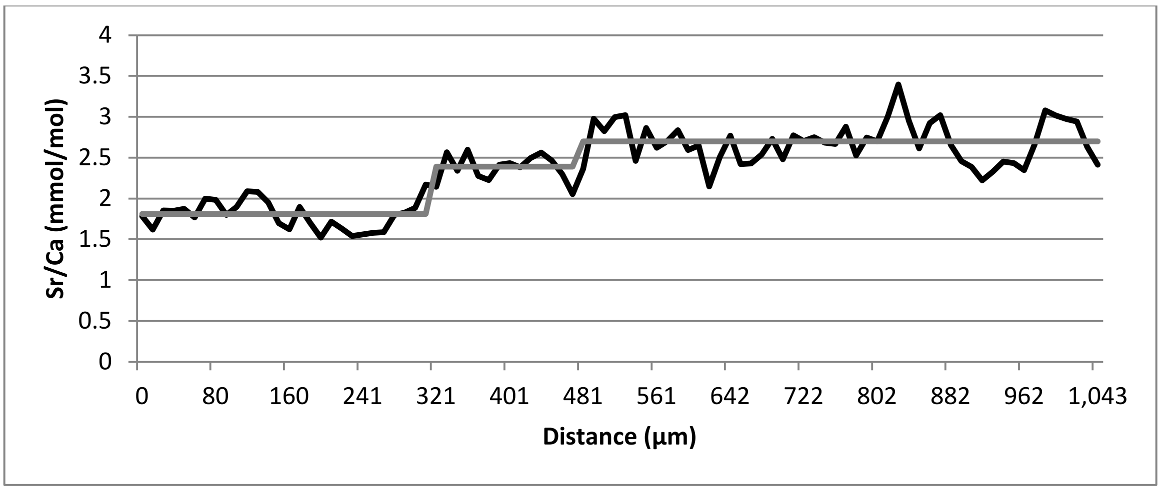
B.


C.

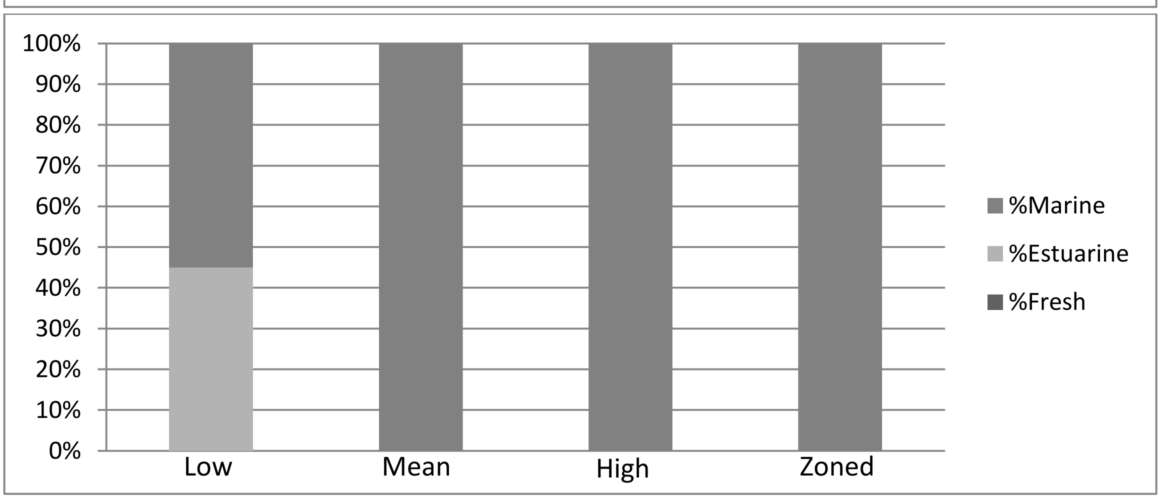
Figure AB.69. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 100. Figure 69.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

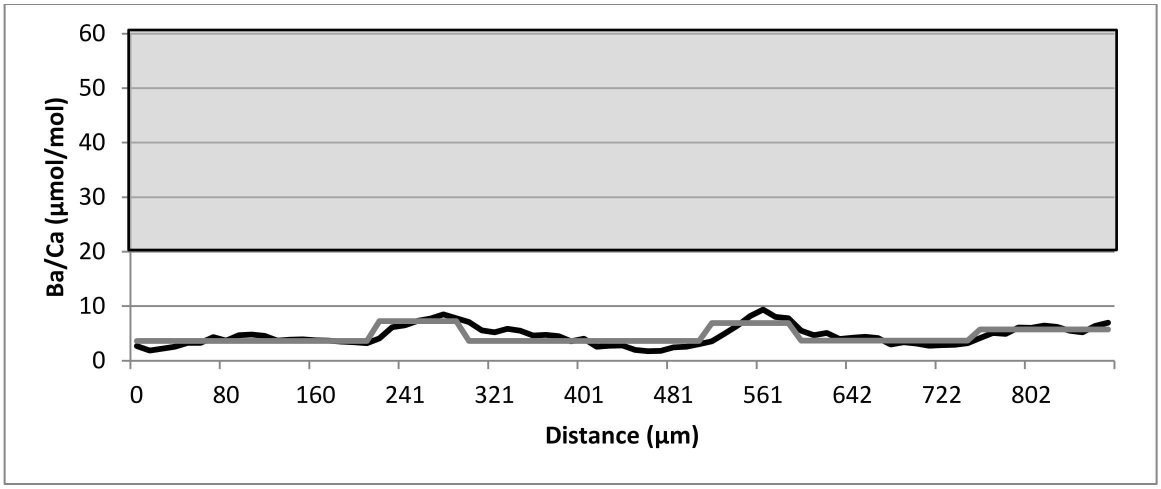


B.

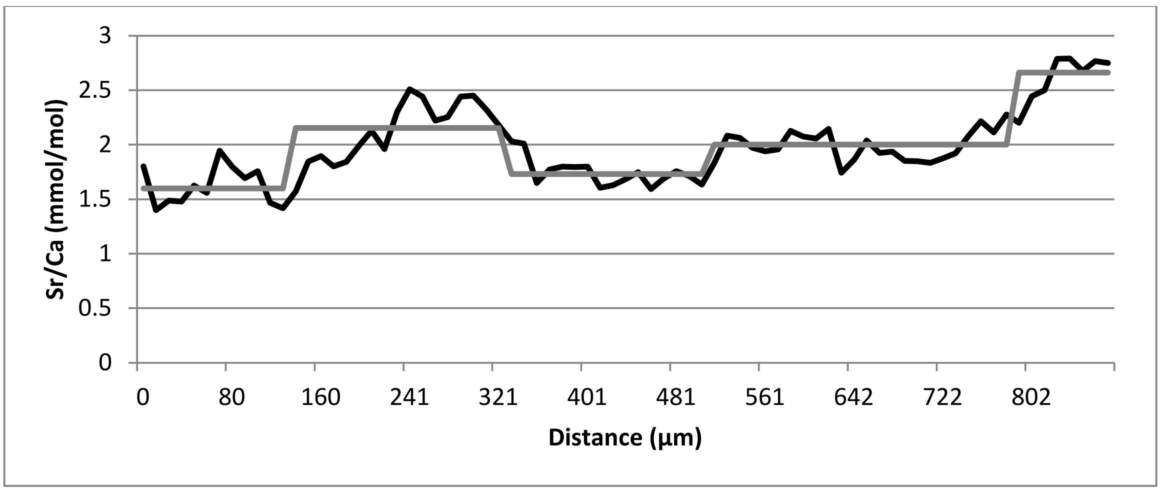


C.

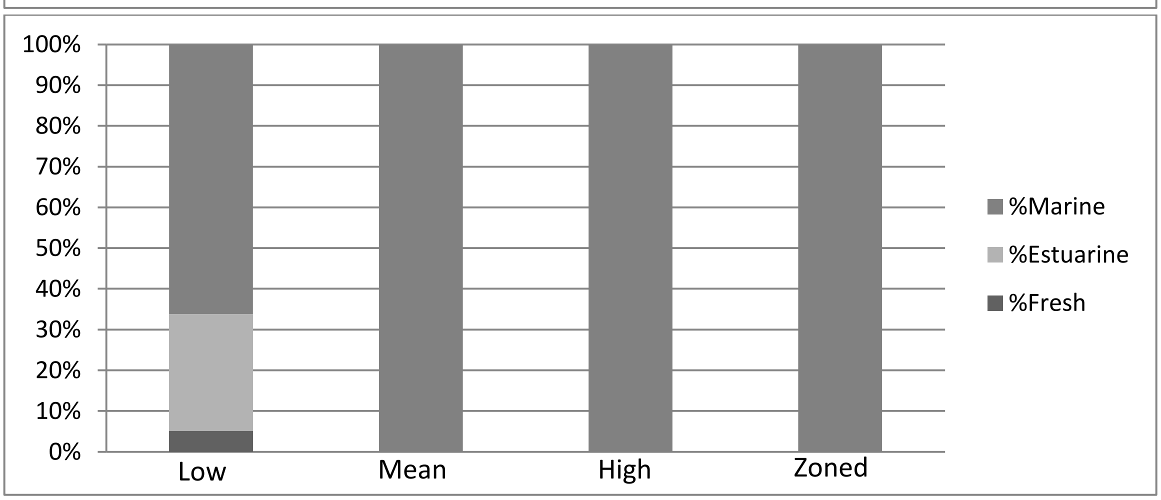
Figure AB.70. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 102. Figure 70.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.71. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 103. Figure 71.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

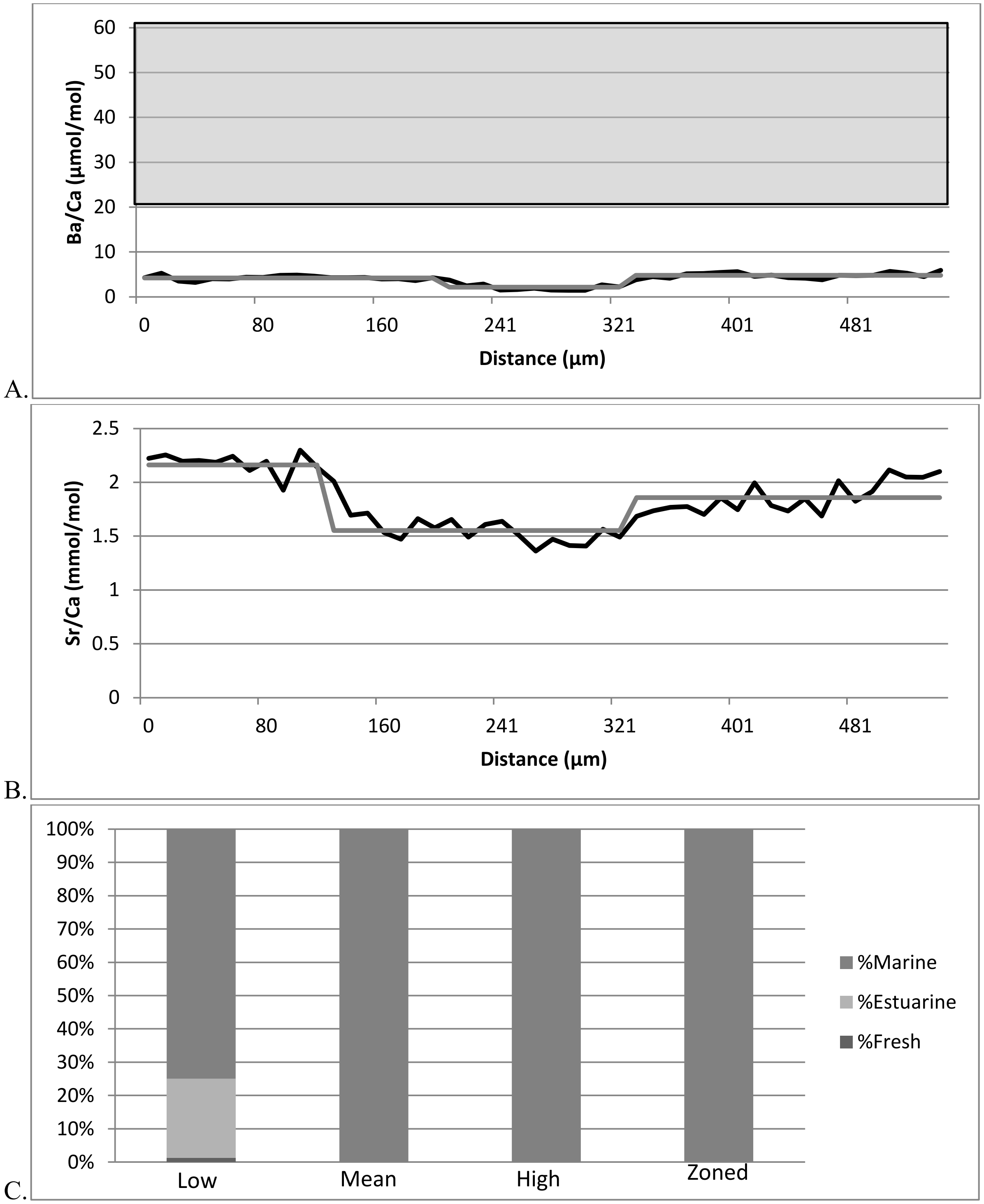


Figure AB.72. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 104. Figure 72.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

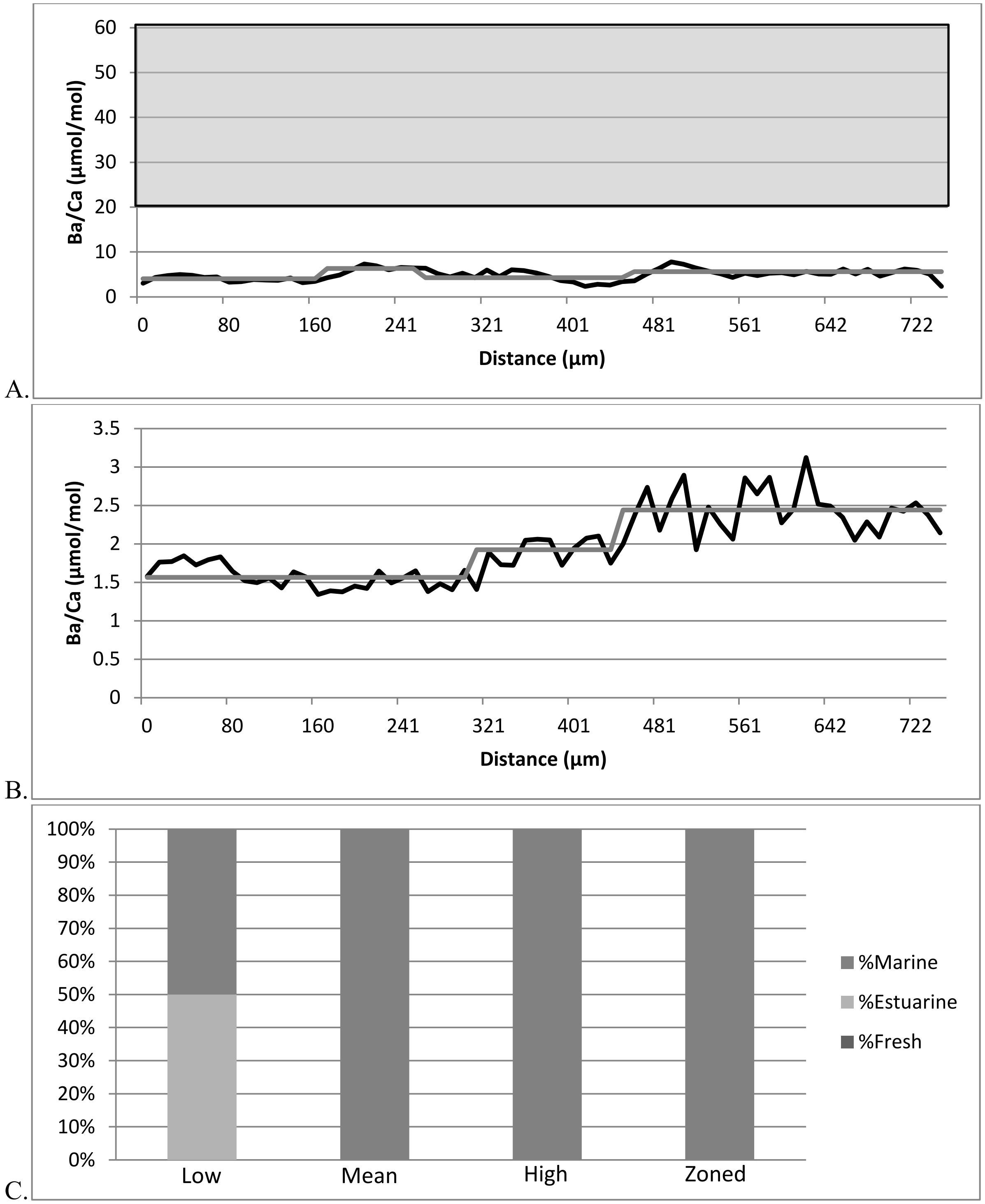
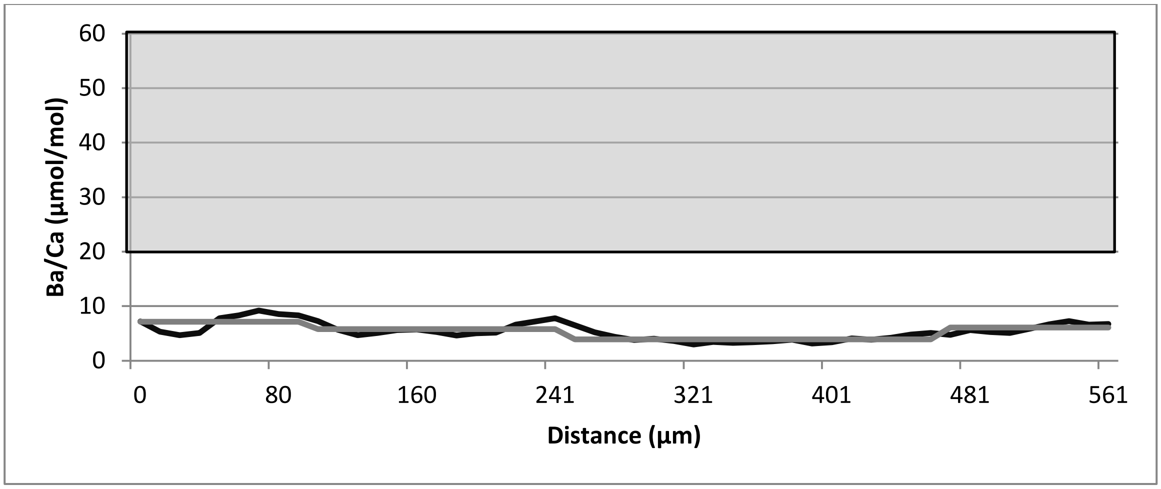
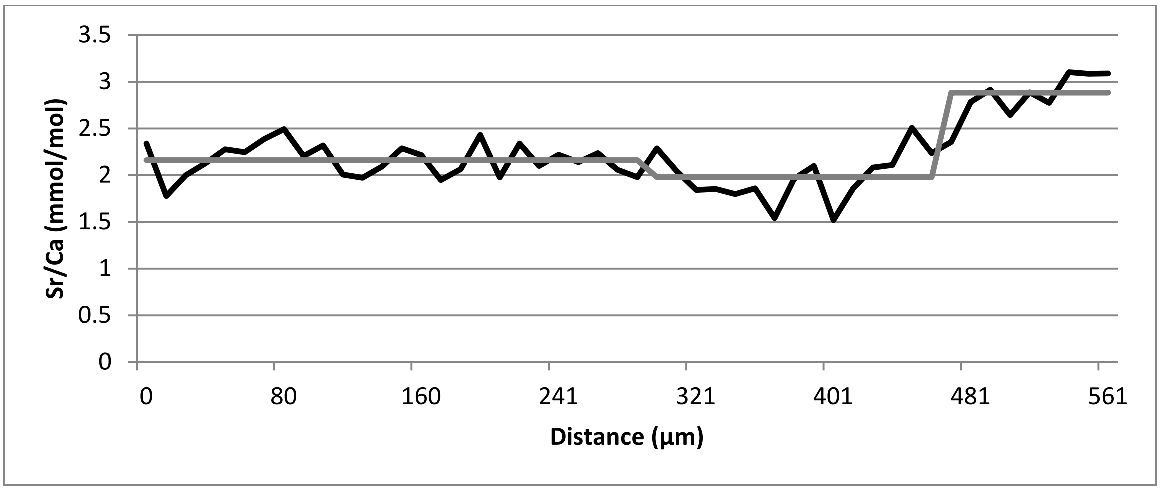


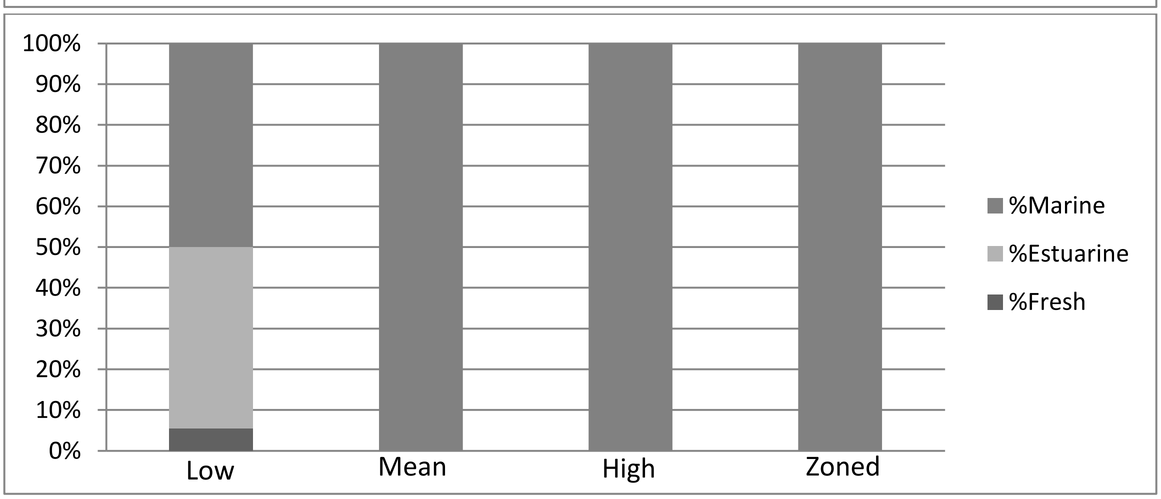
Figure AB.73. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 105. Figure 73.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

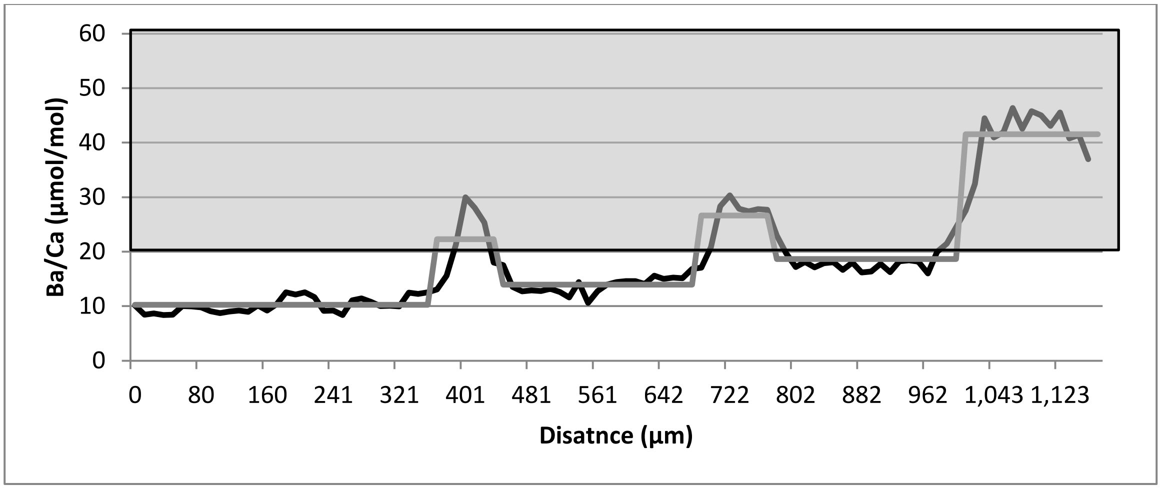


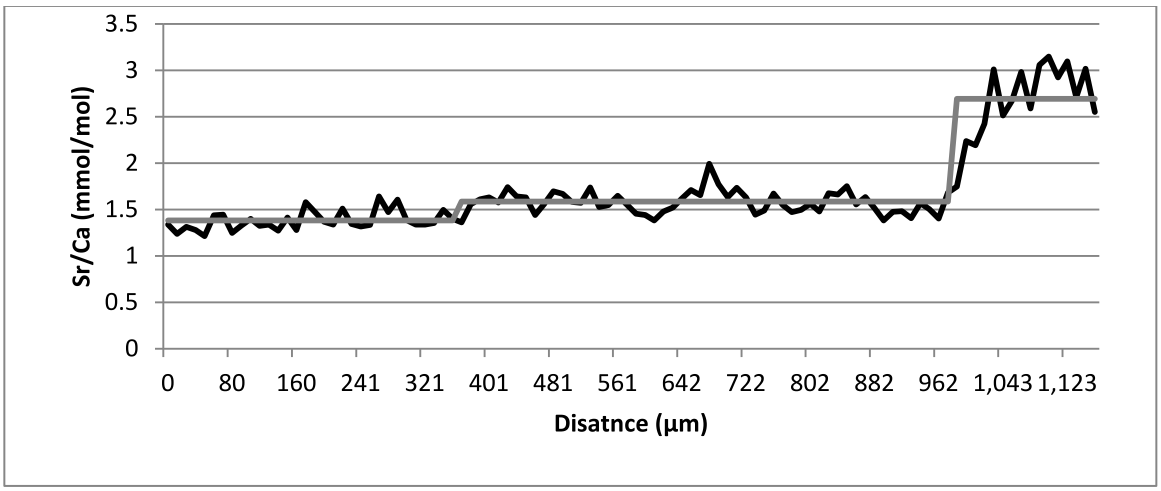
B.



C.

Figure AB.74. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 106. Figure 74.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


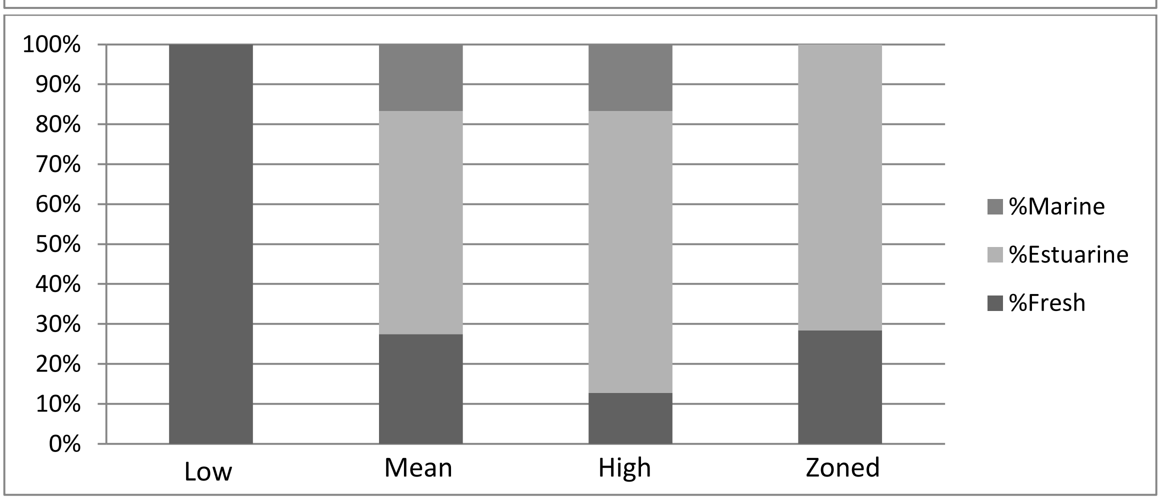
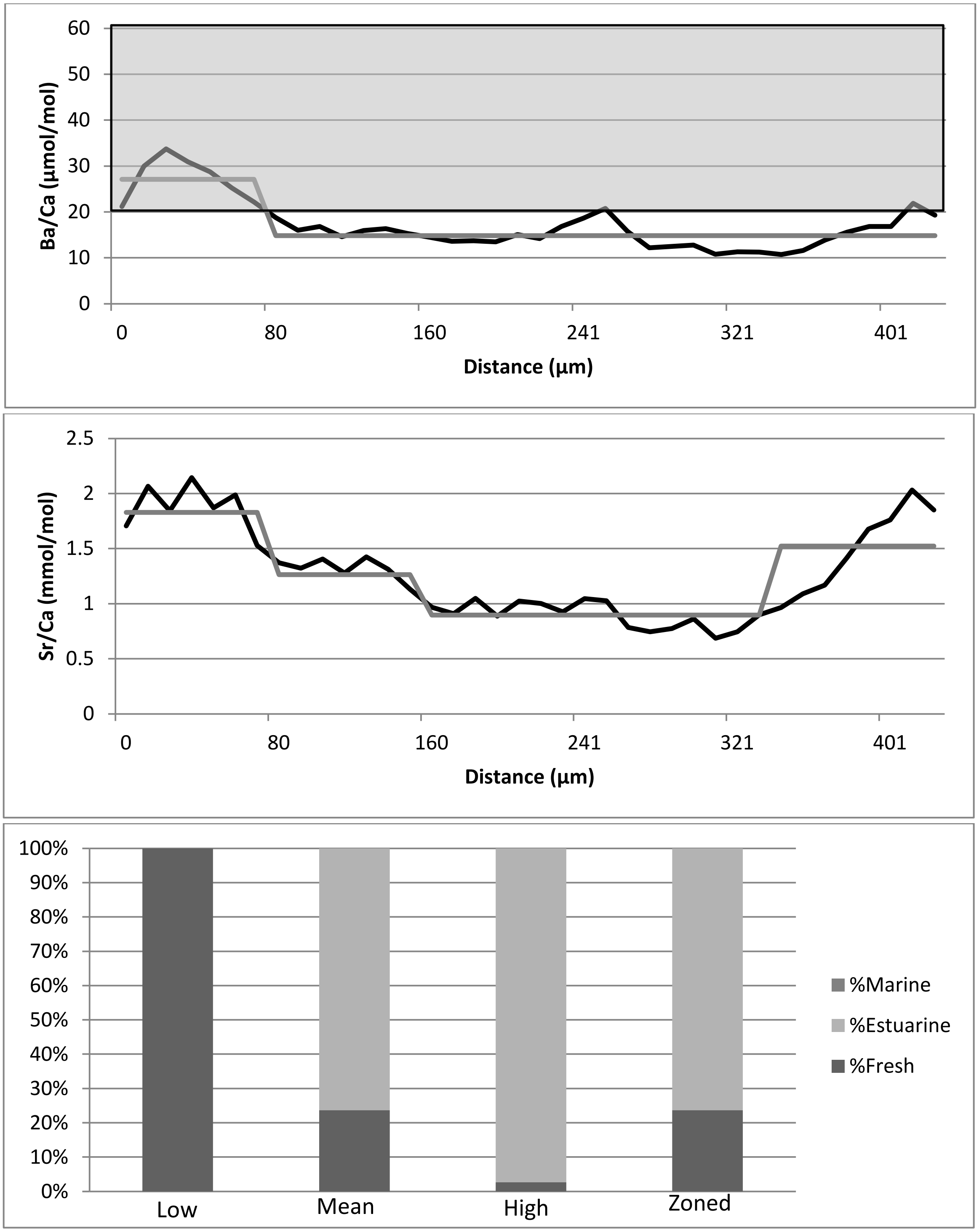
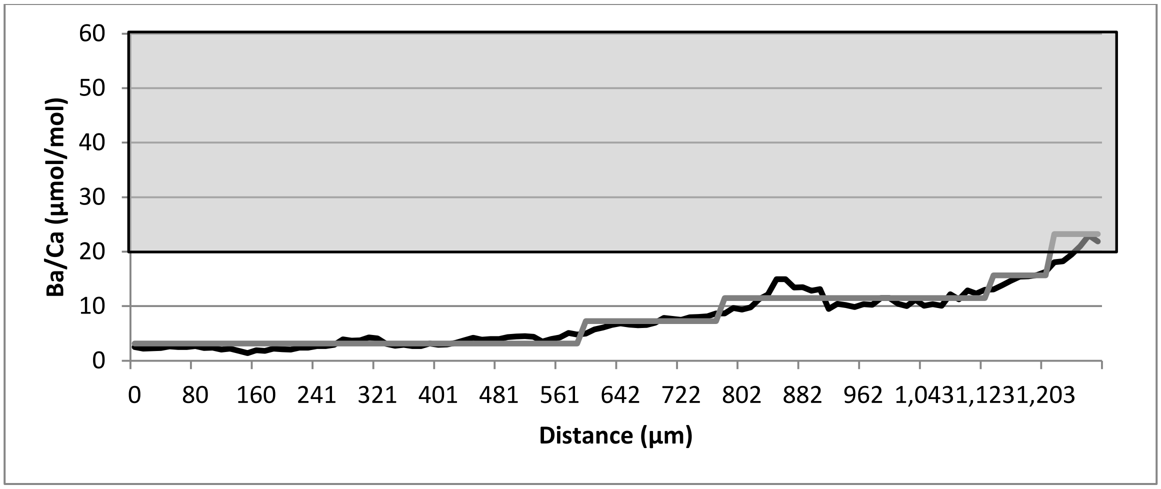
C.


Figure AB.75. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 110. Figure 75.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

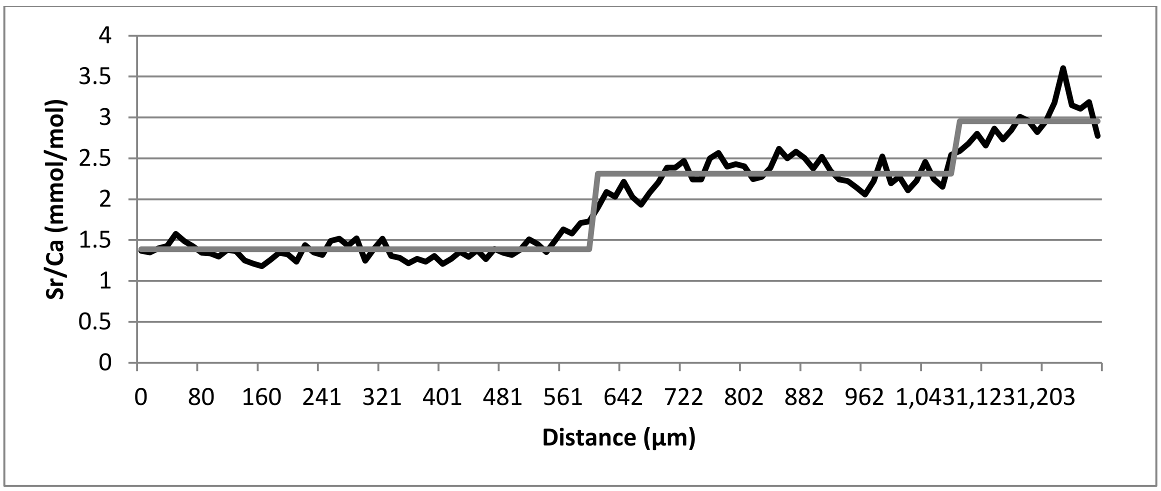


A.
B.
C.

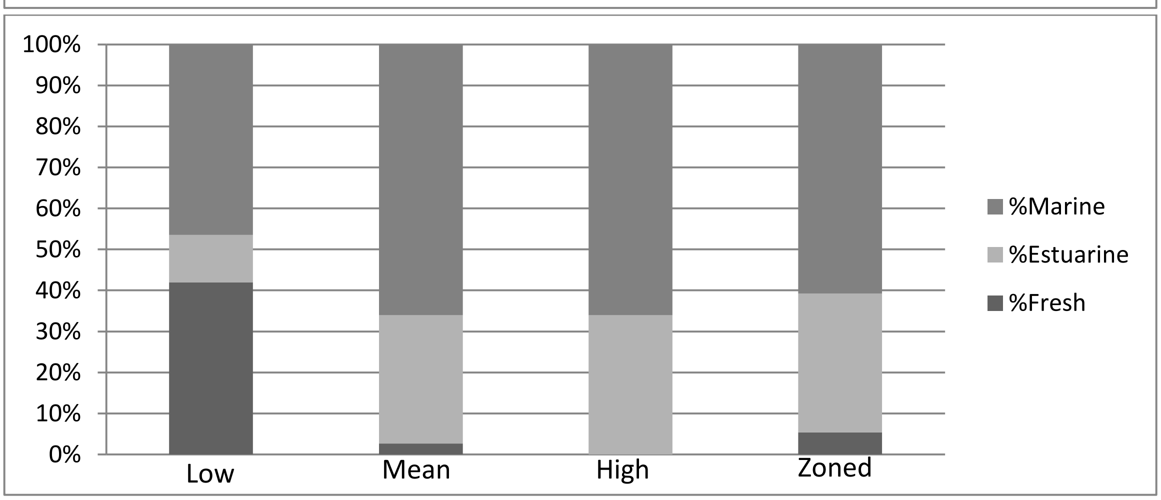
Figure AB.76. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 111. Figure 76.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

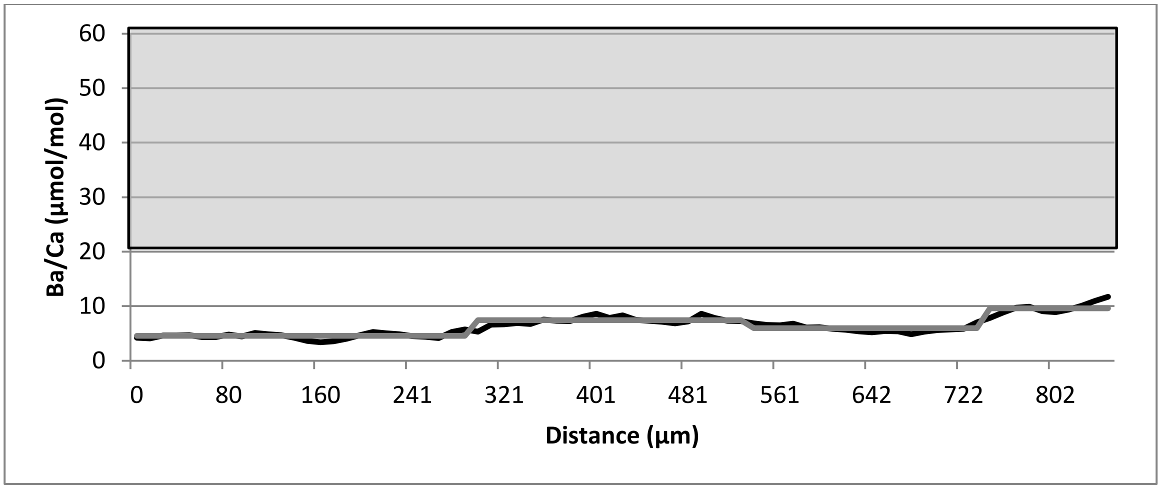


B.

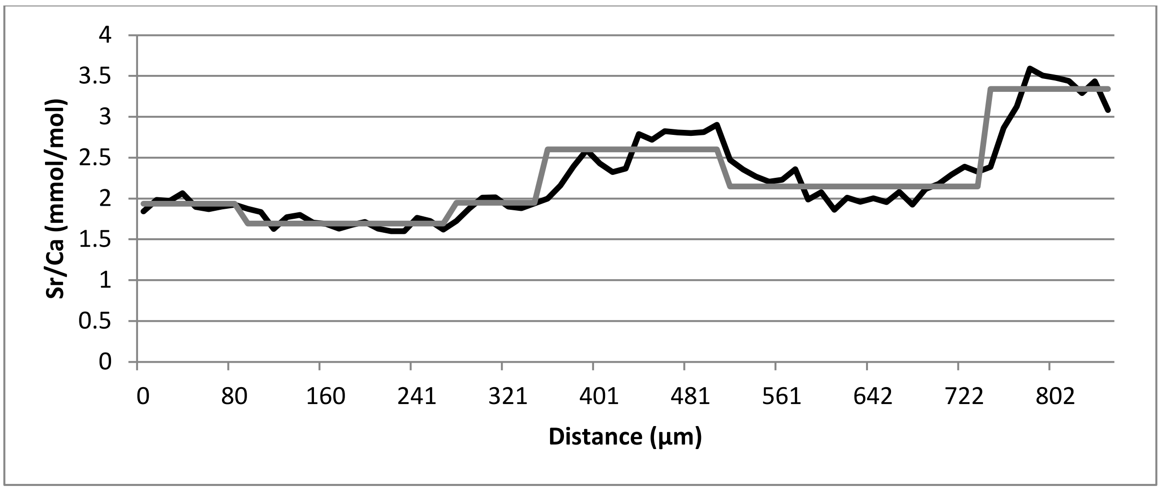


C.

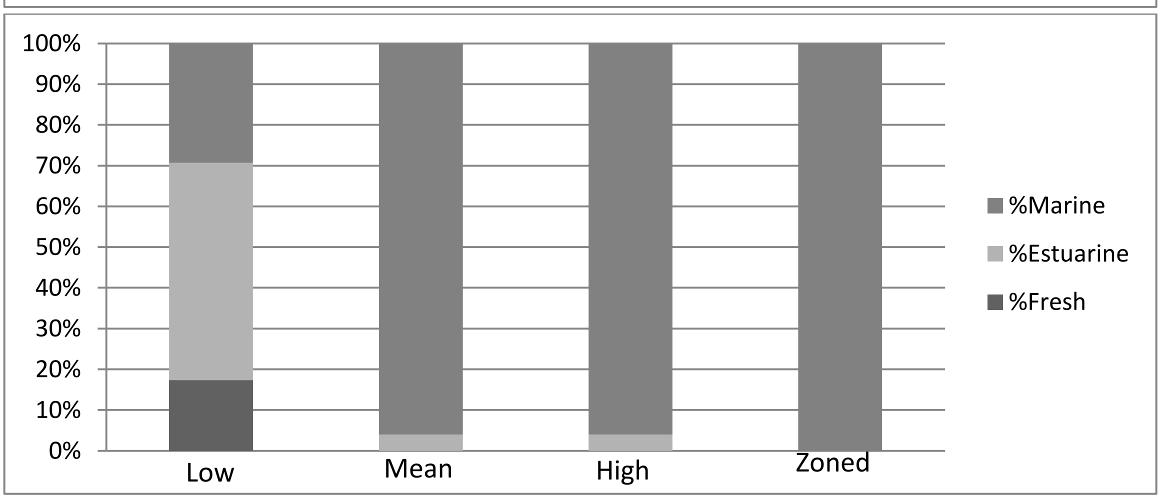
Figure AB.77. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 112. Figure 77.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

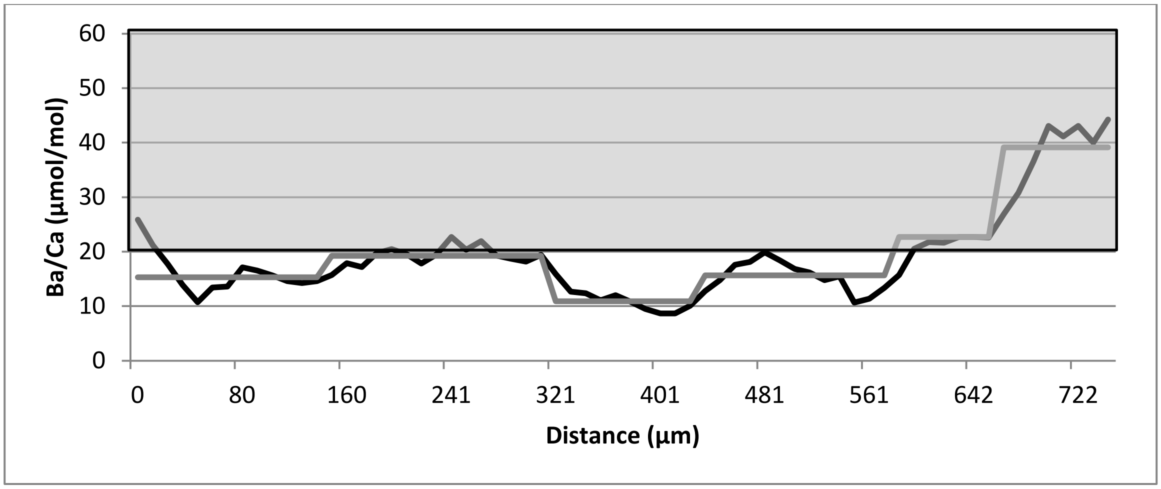


B.

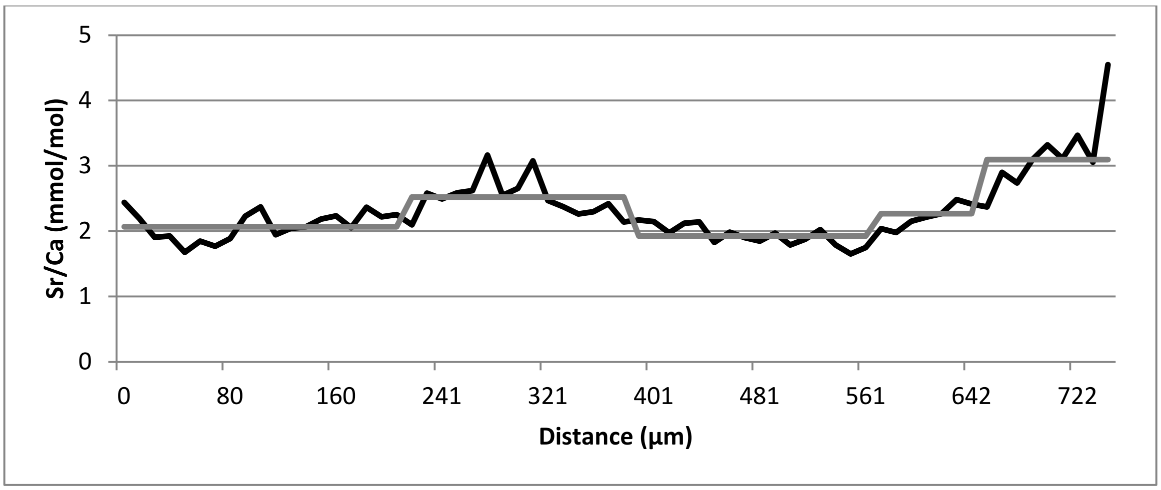


C.

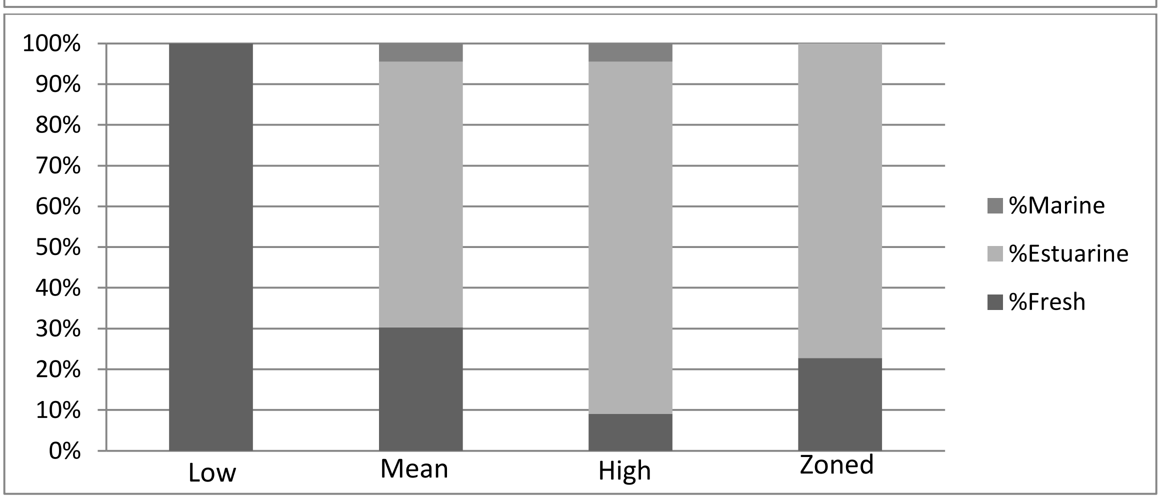
Figure AB.78. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 113. Figure 78.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

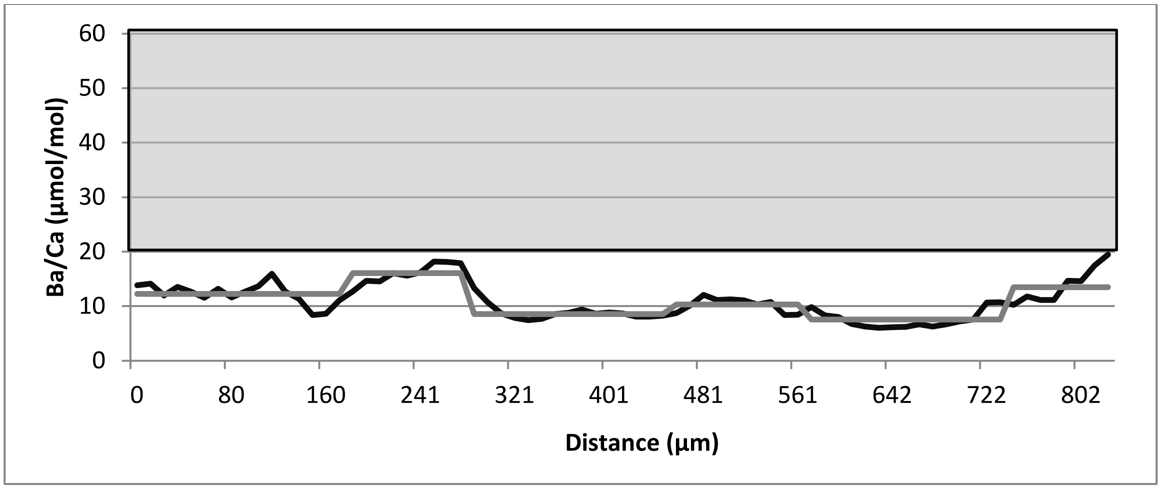


B.

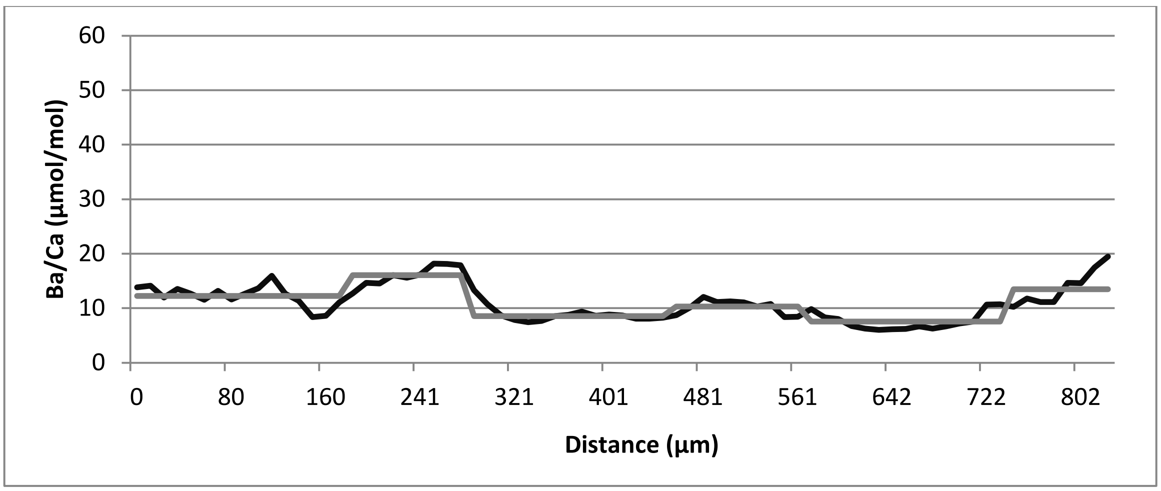


C.

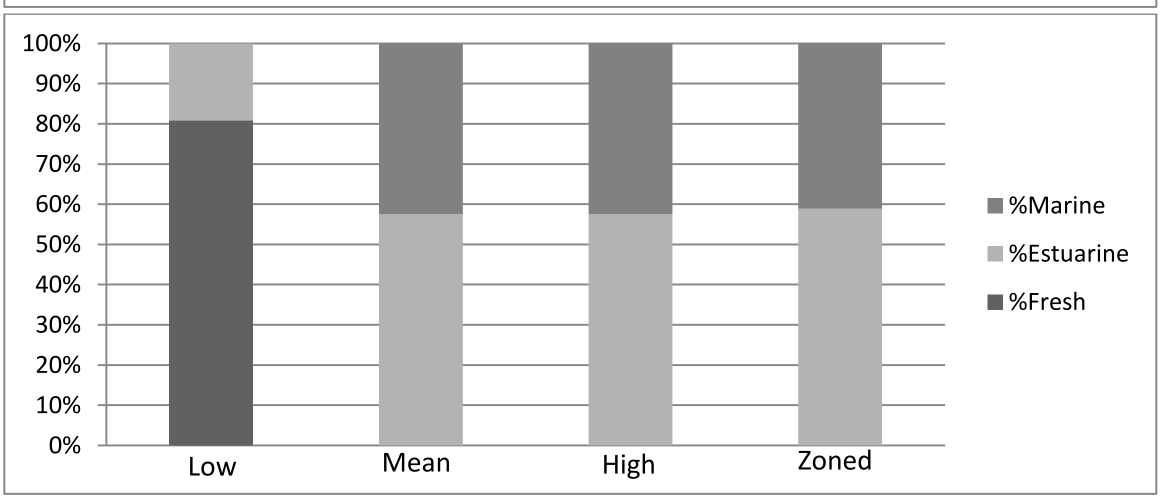
Figure AB.79. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 114. Figure 79.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

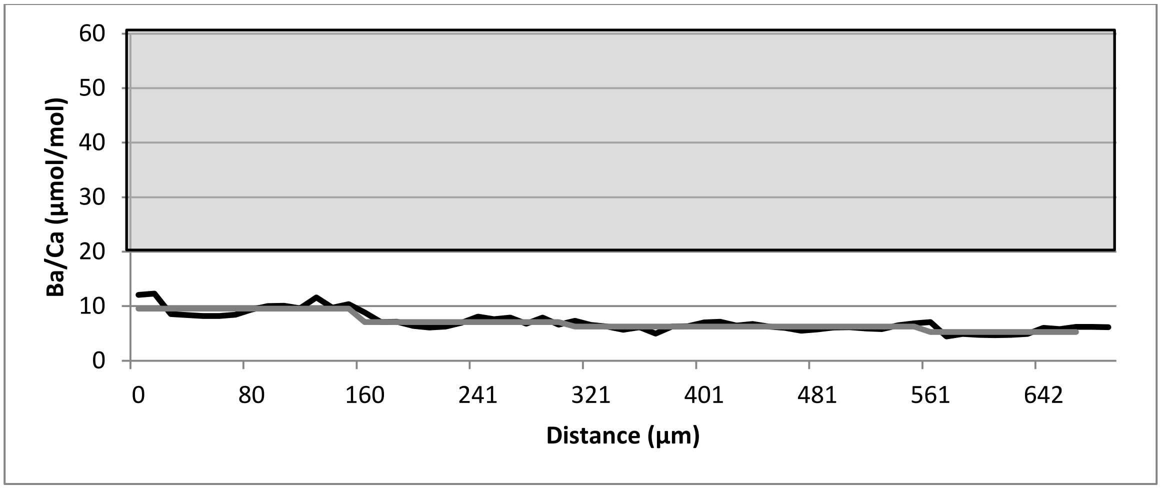


B.

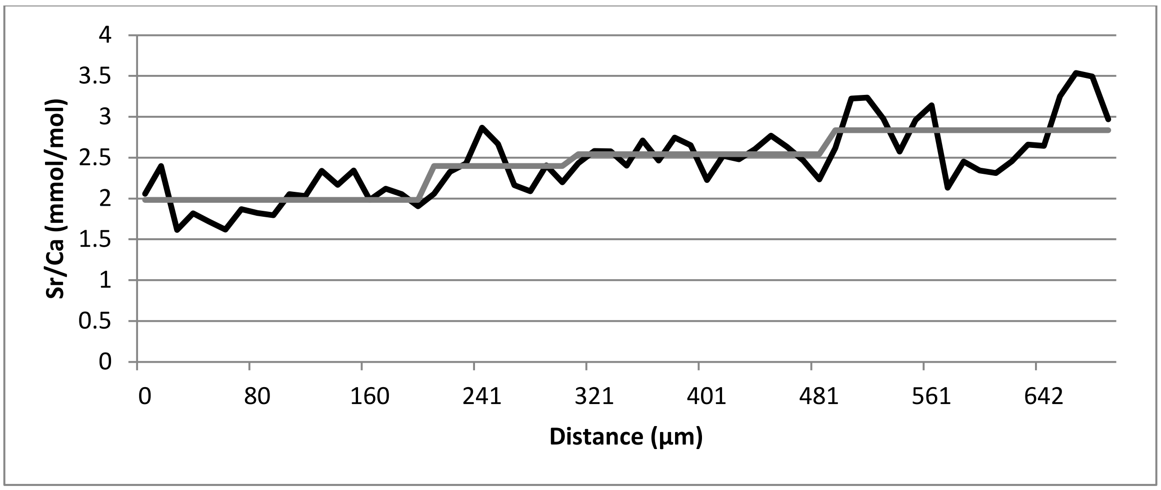


C.

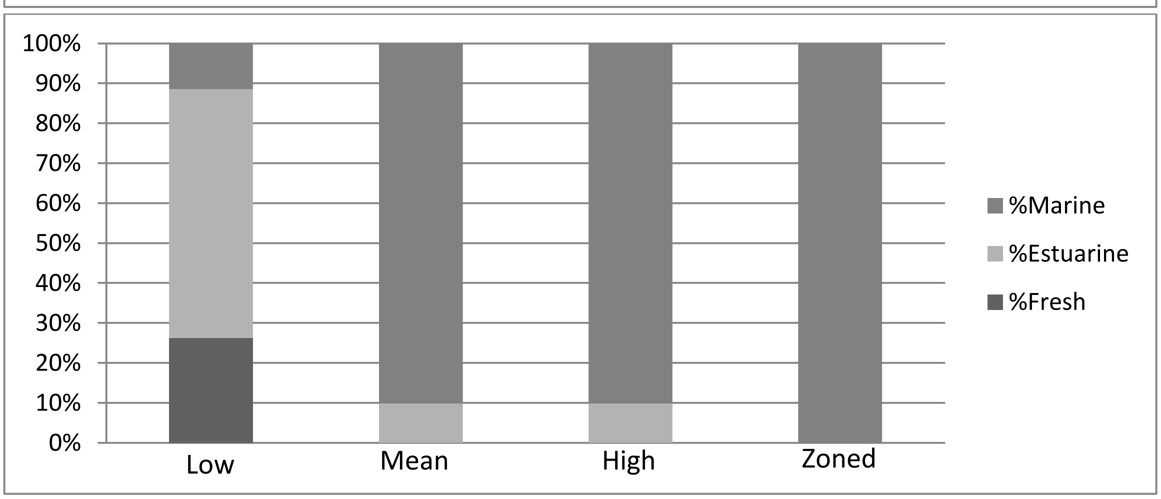
Figure AB.80. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 115. Figure 80.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



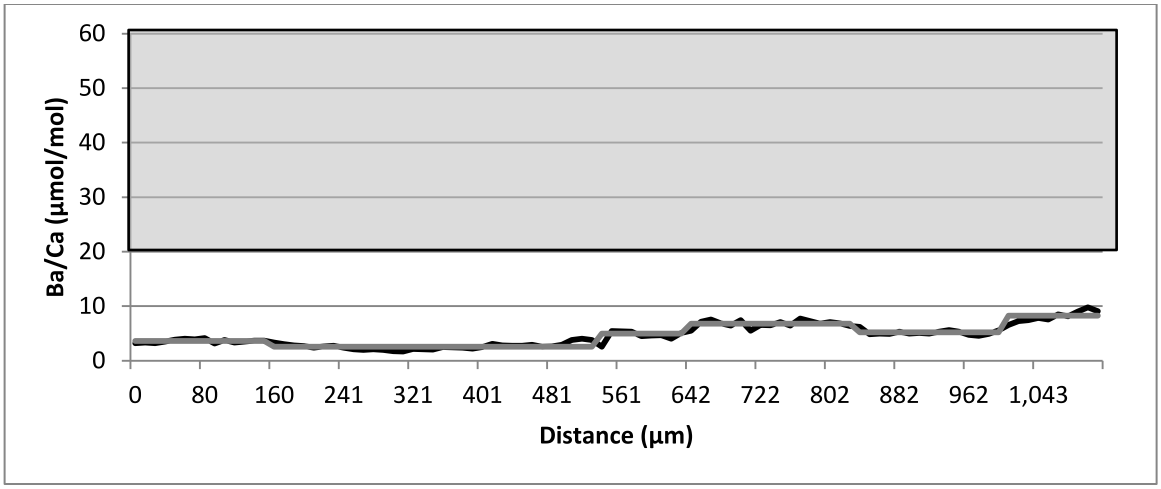
B.



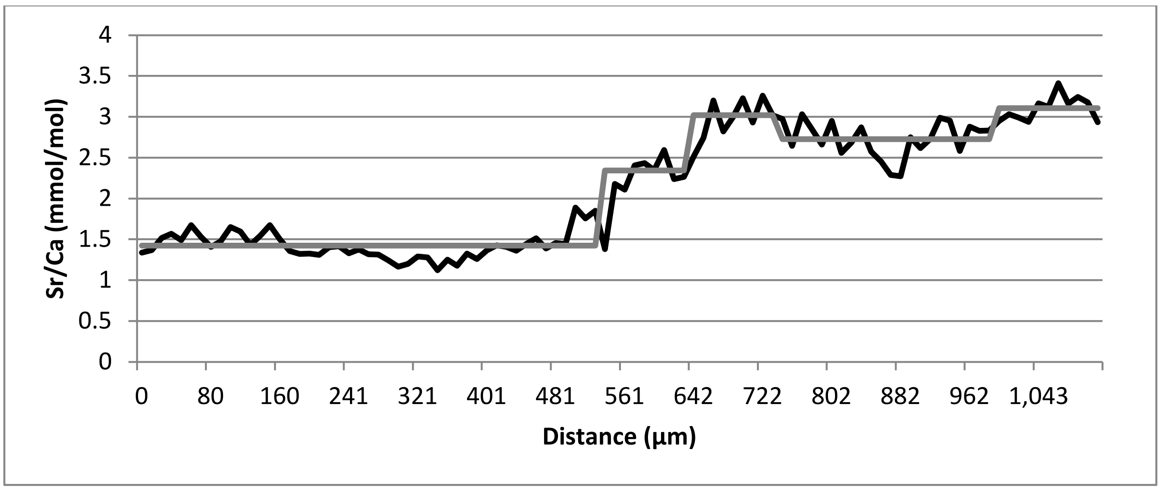
C.

Figure AB.81. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 116. Figure 81.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.



B.



C.

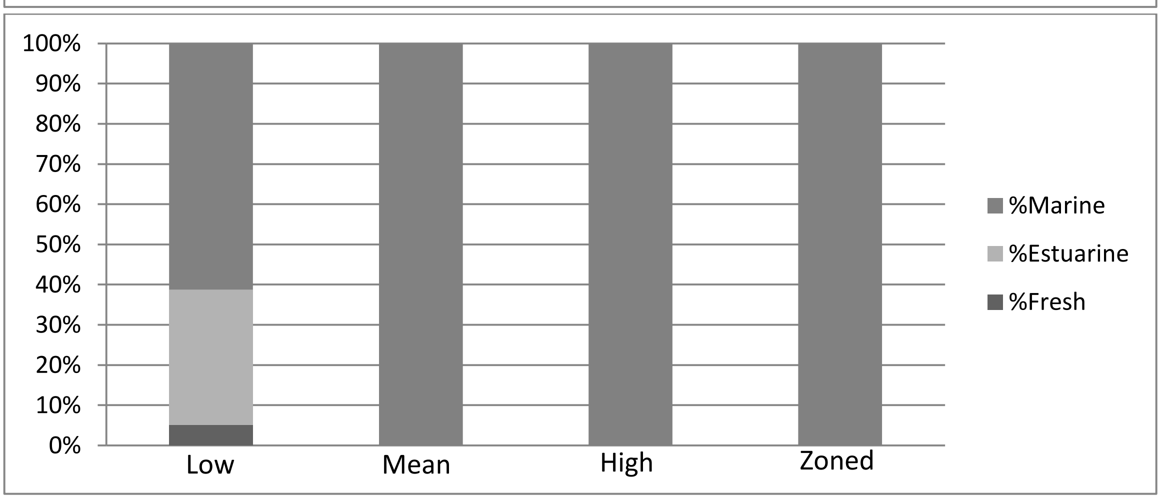
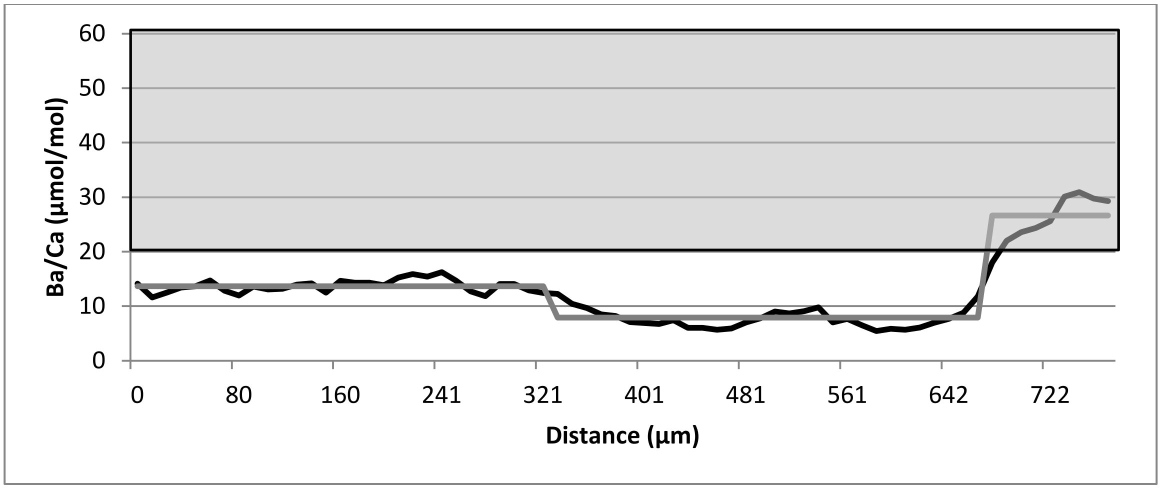
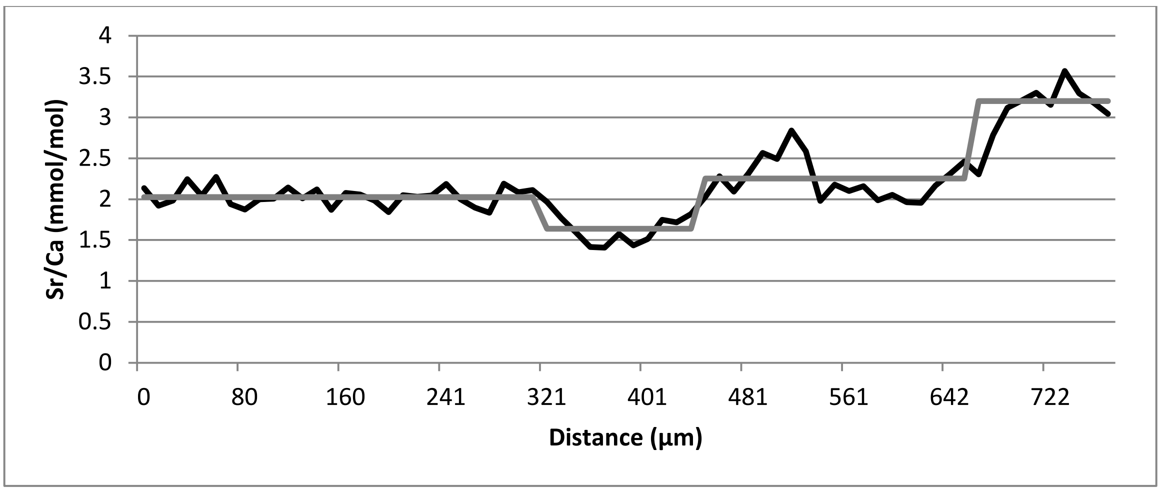


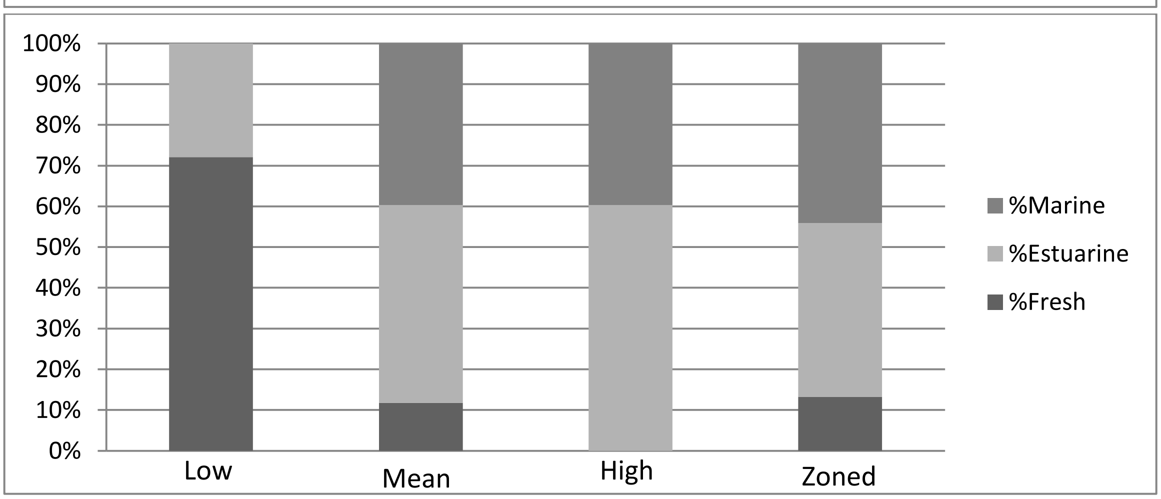
Figure AB.82. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 117. Figure 82.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

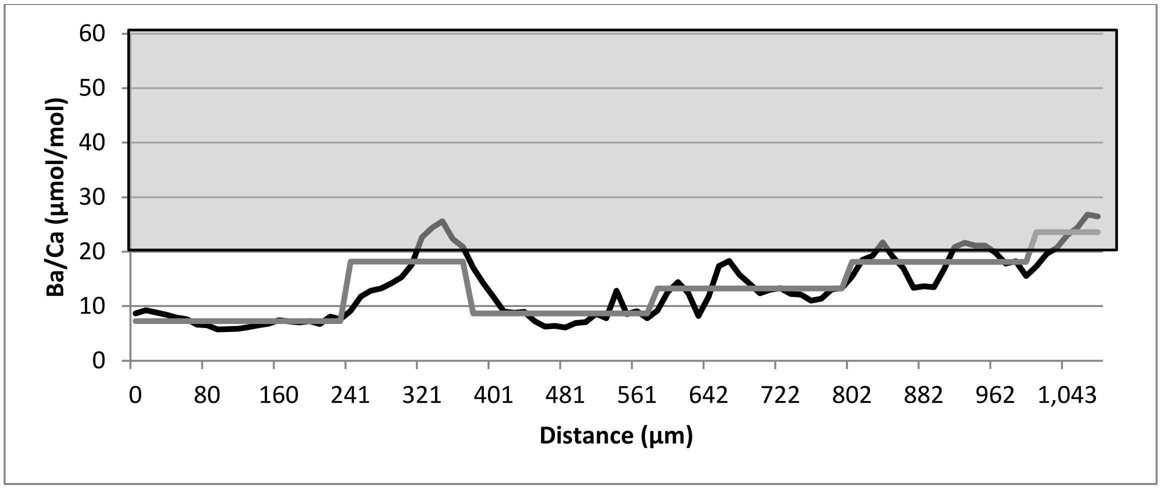


B.

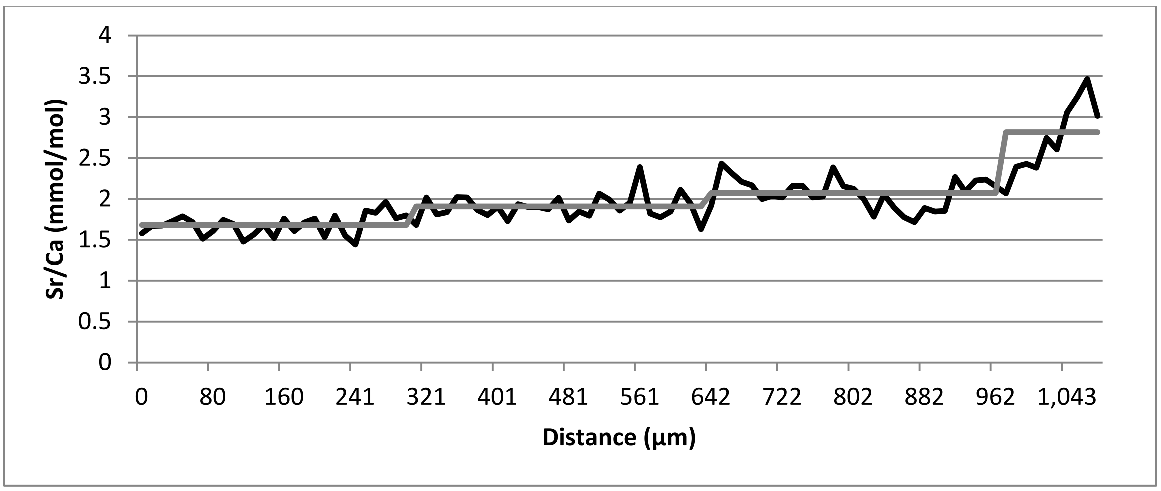


C.

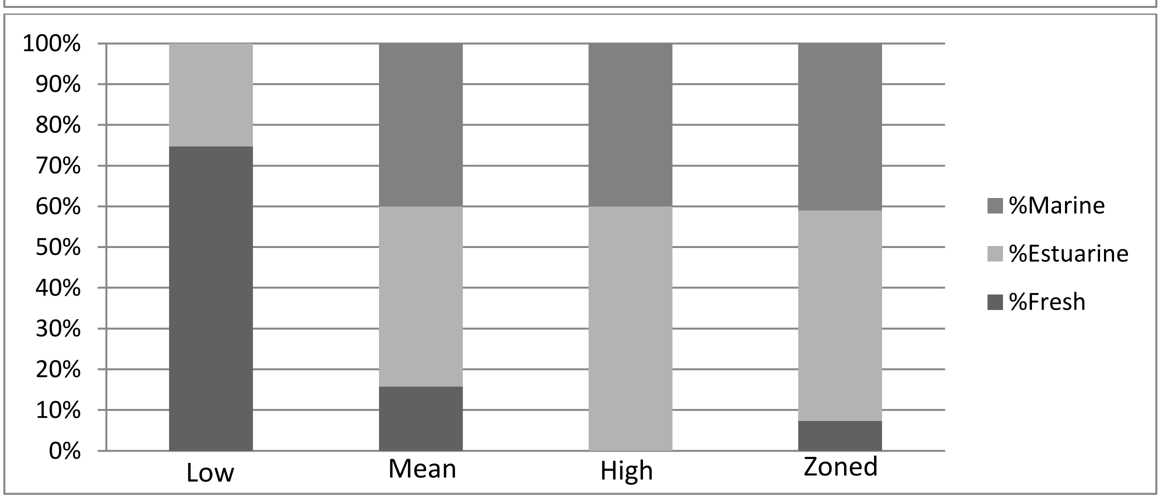
Figure AB.83. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 118. Figure 83.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

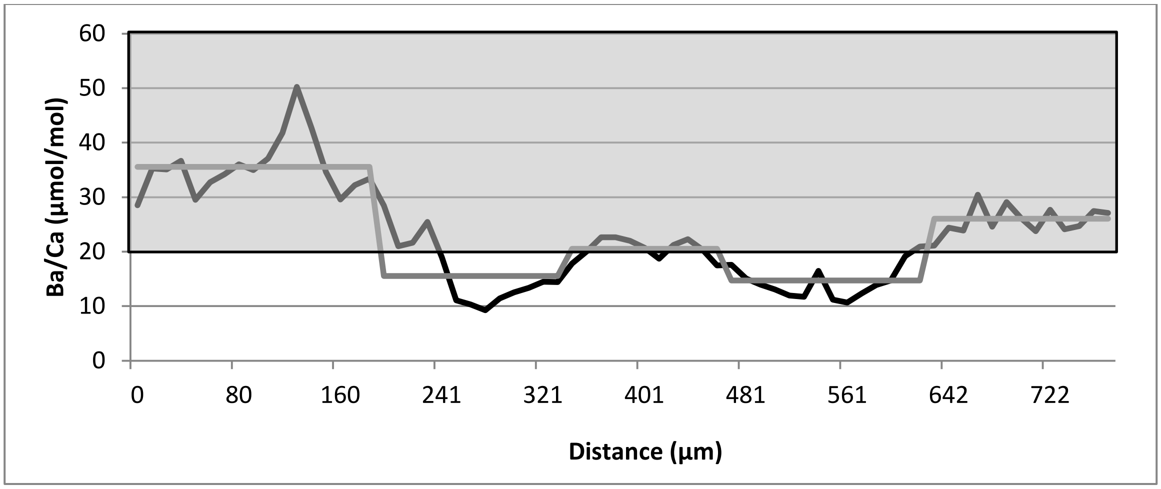


B.

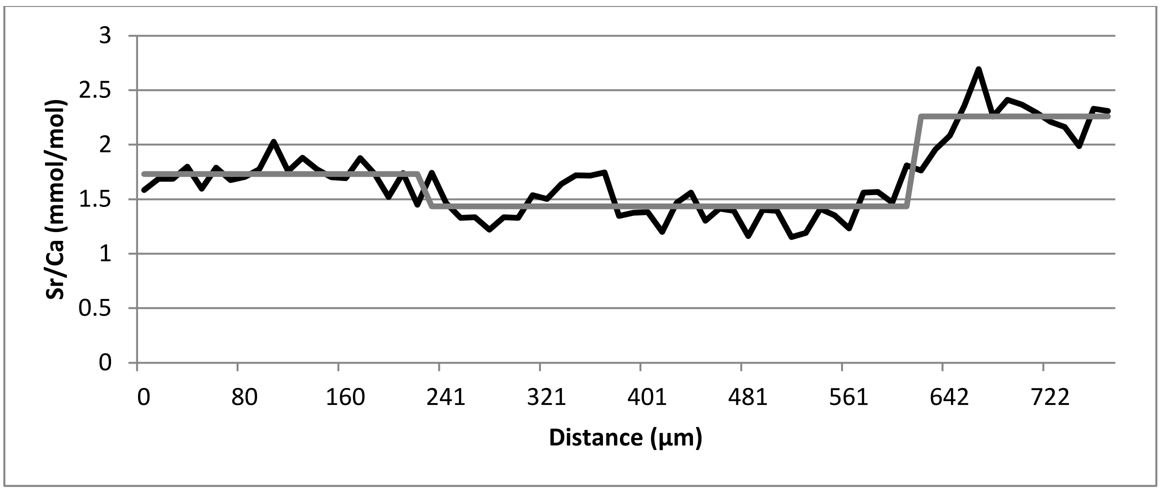


C.

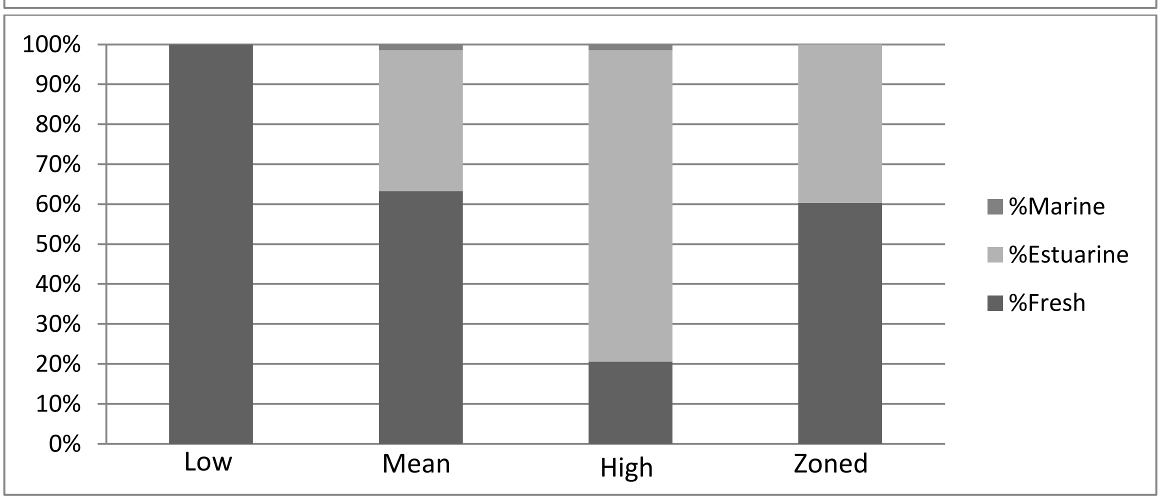
Figure AB.84. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 119. Figure 84.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.85. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 120. Figure 85.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

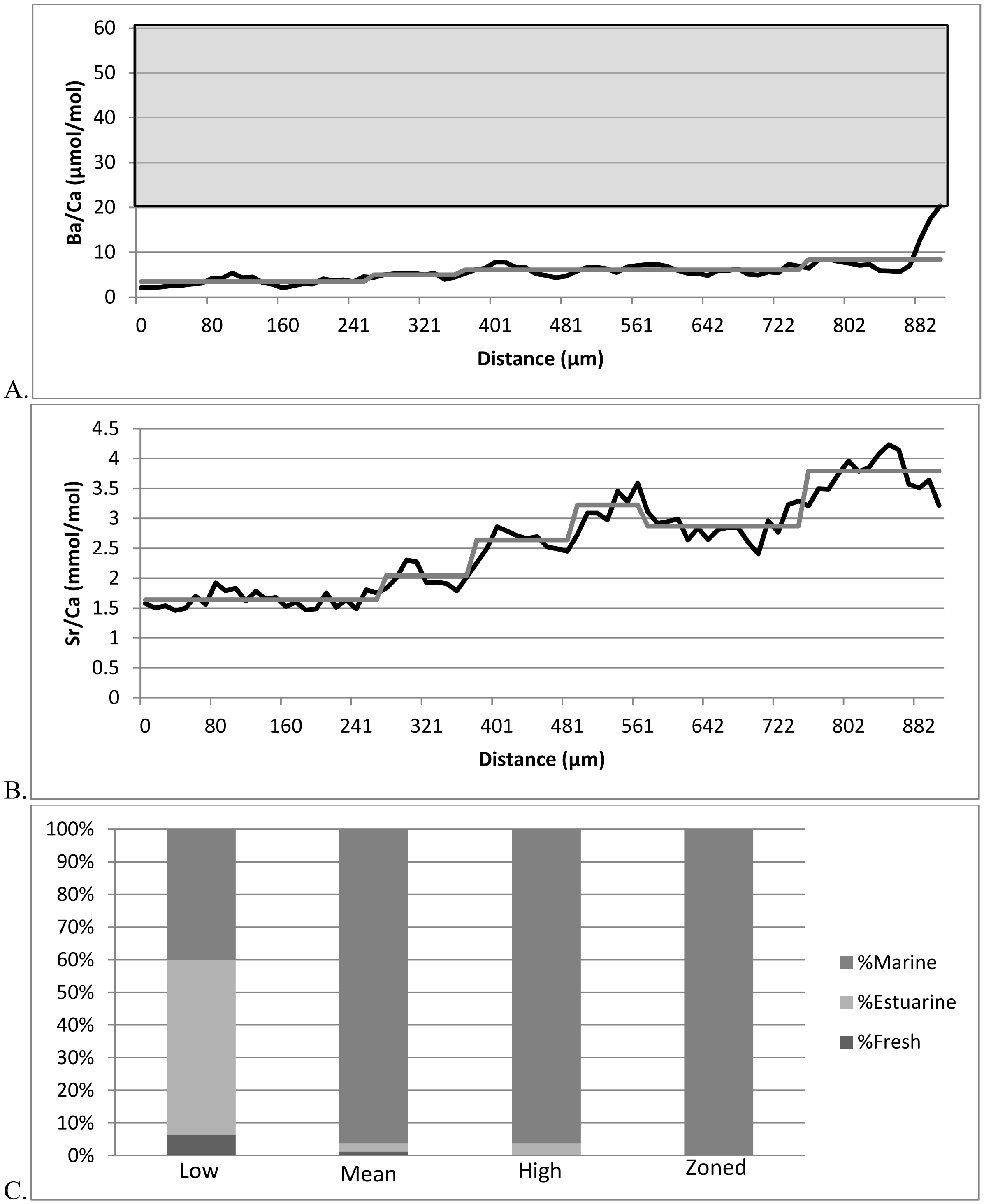
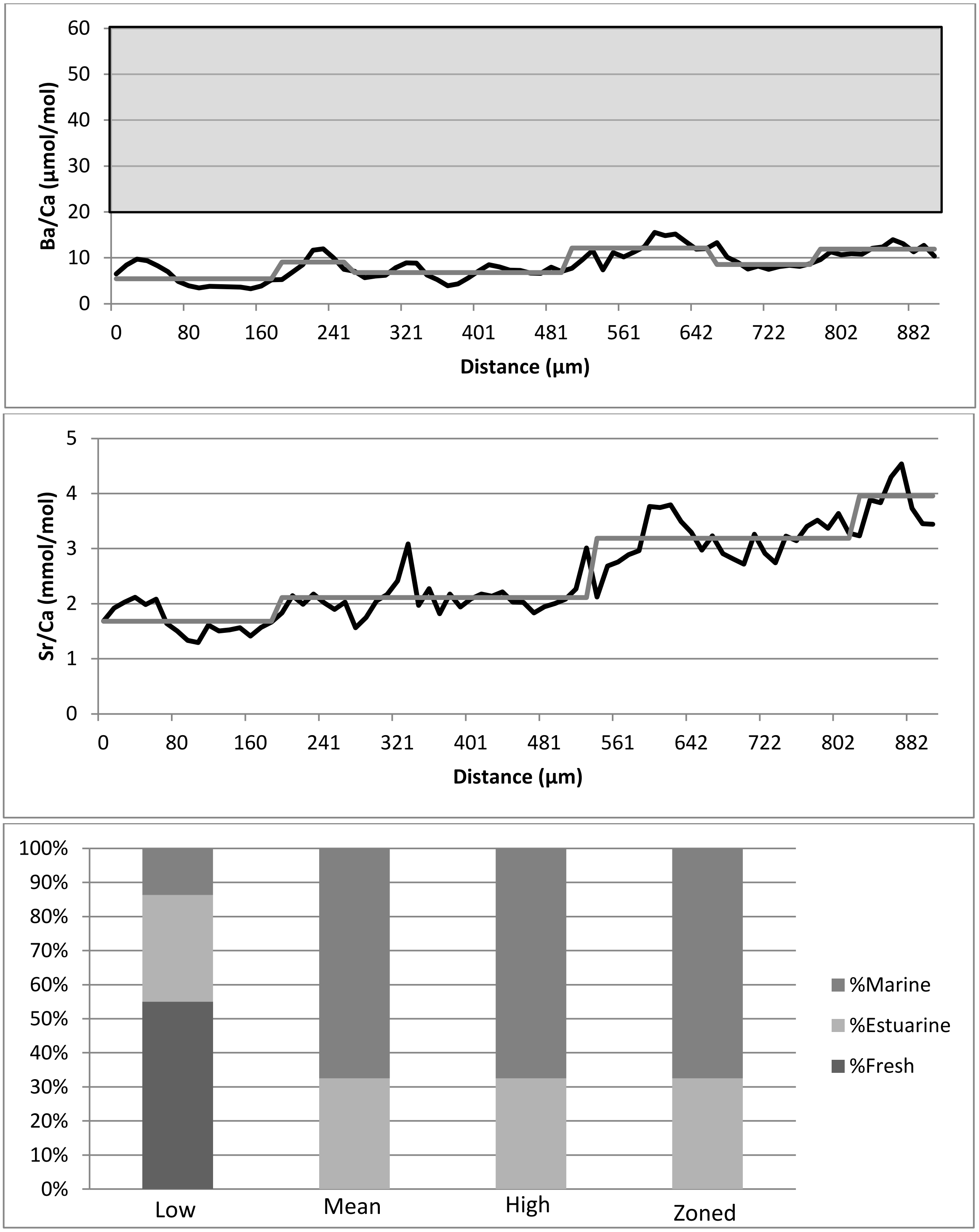


Figure AB.86. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 121. Figure 86.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

B.

C.

Figure AB.87. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 122. Figure 87.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

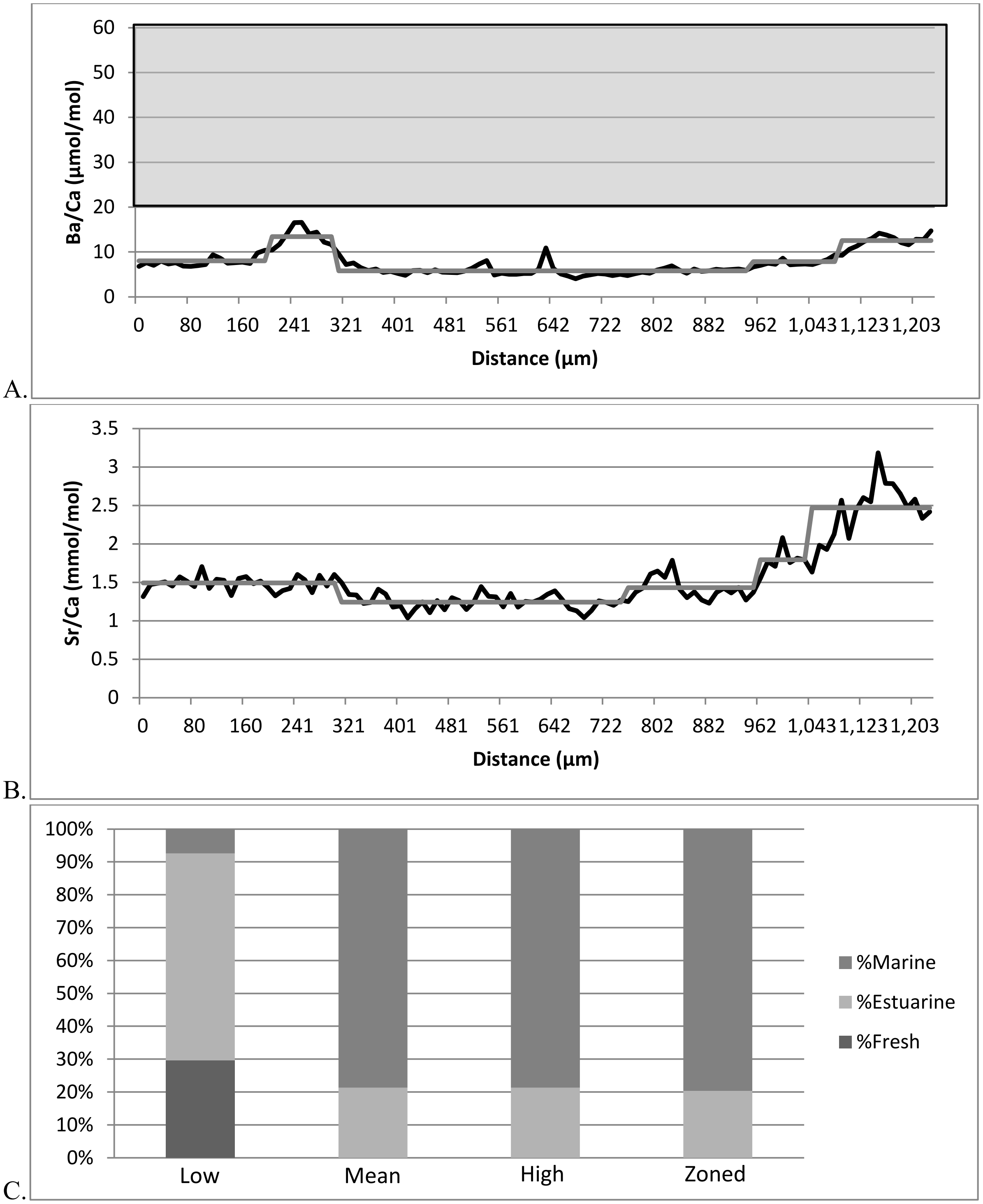
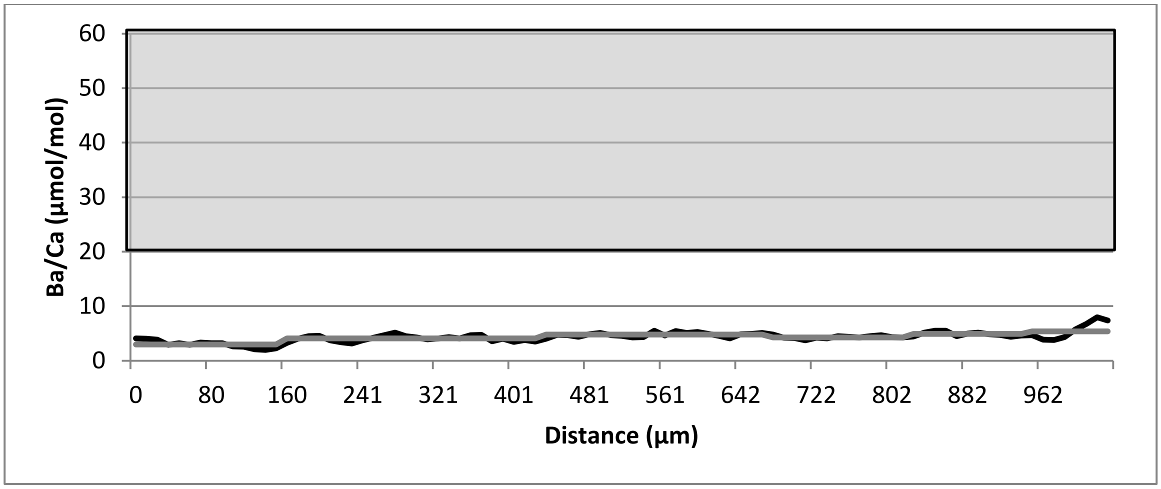
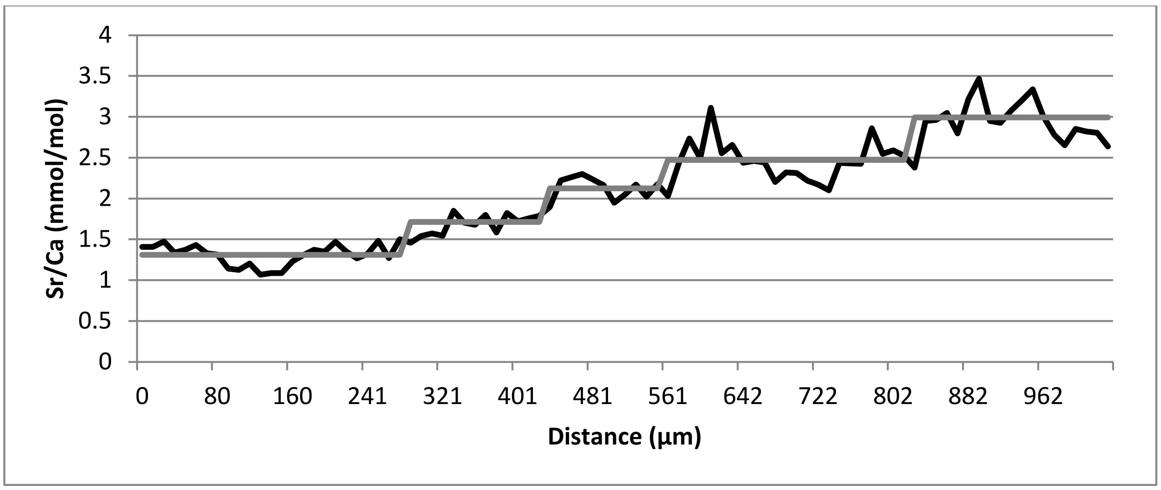


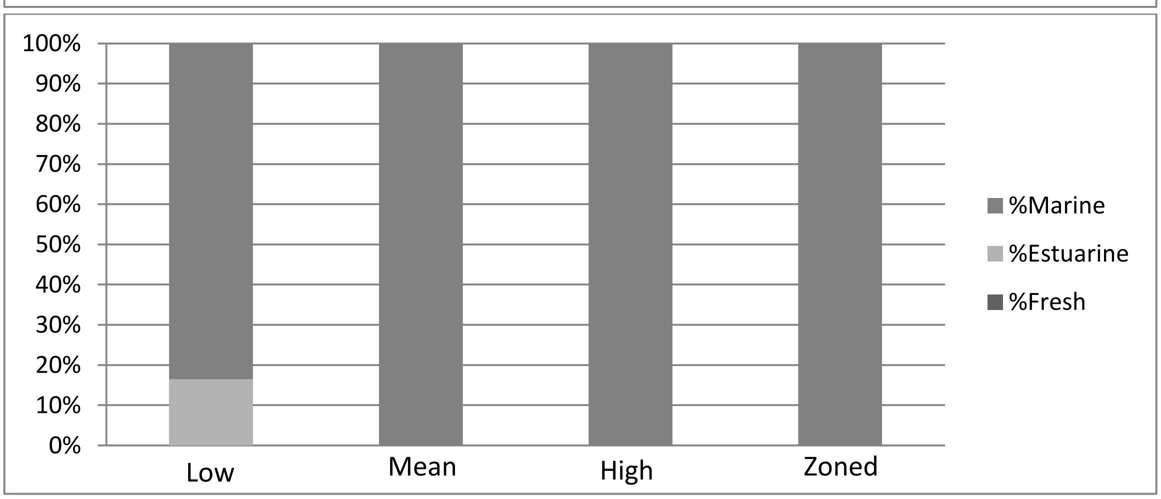
Figure AB.88. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 123. Figure 88.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

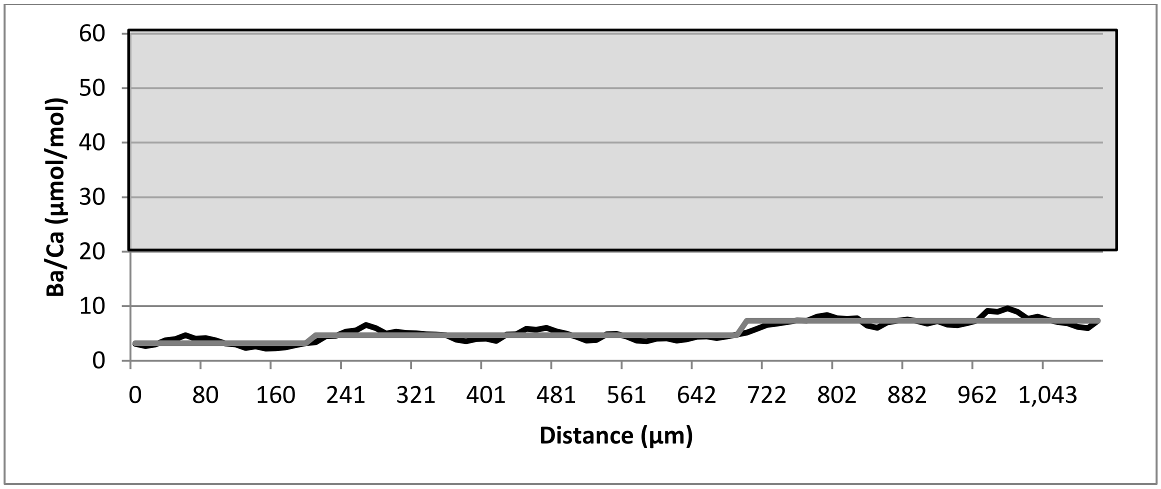


B.

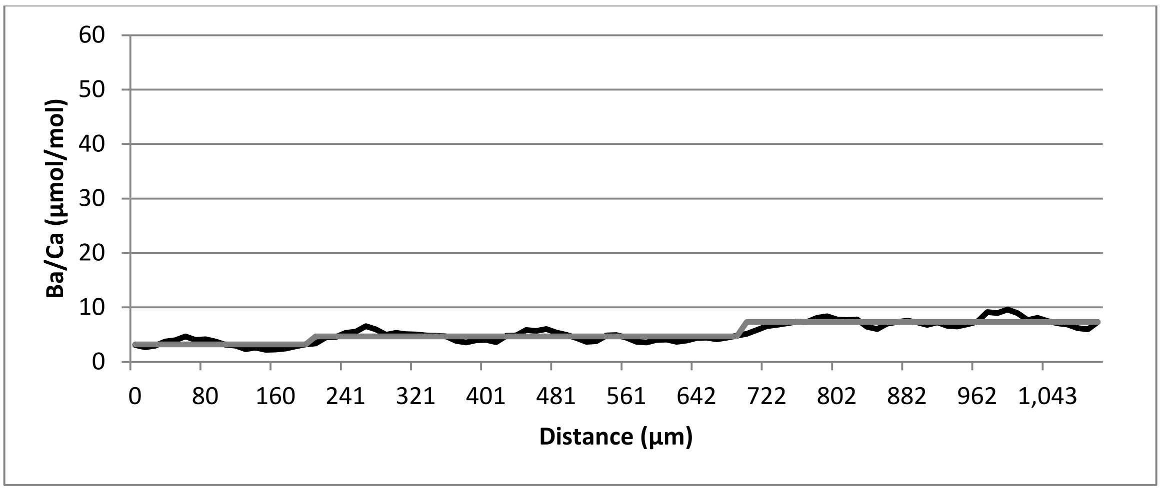


C.

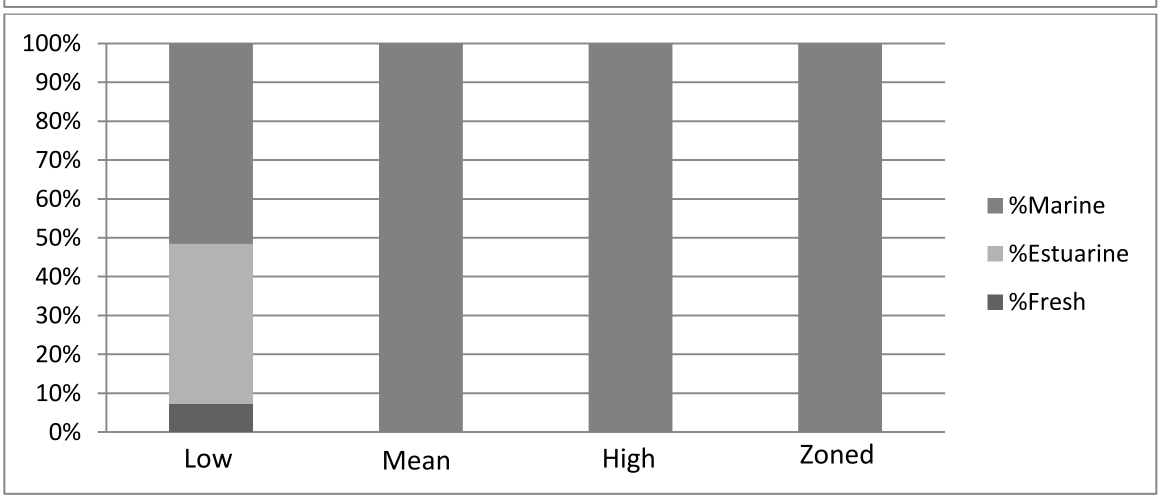
Figure AB.89. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 124. Figure 89.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

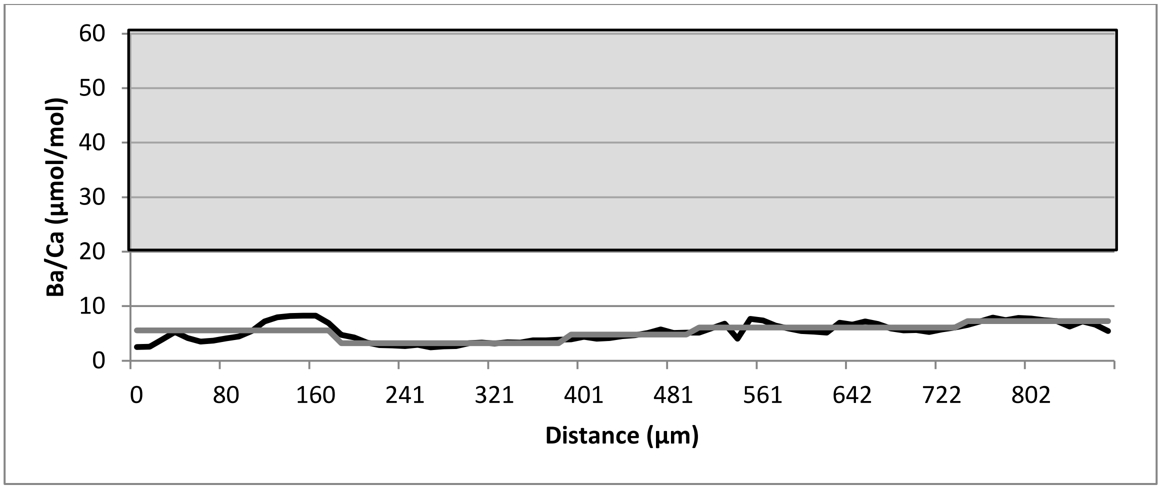


B.

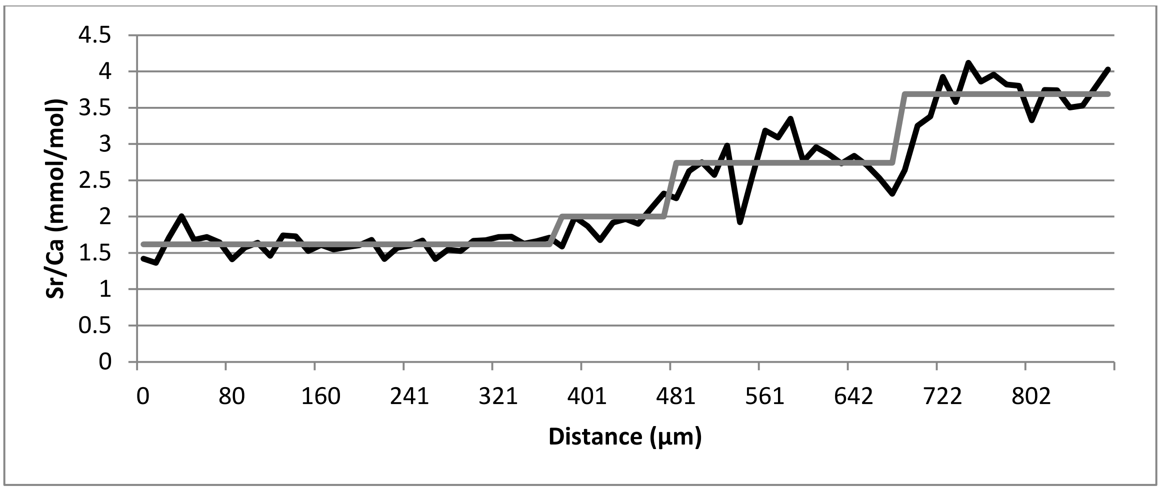


C.

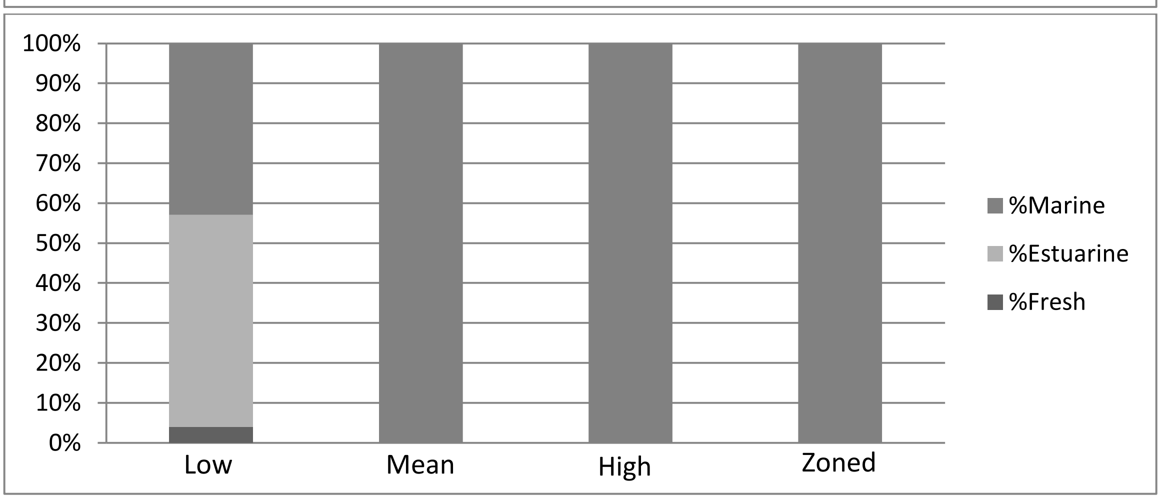
Figure AB.90. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 126. Figure 90.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.91. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 127. Figure 91.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

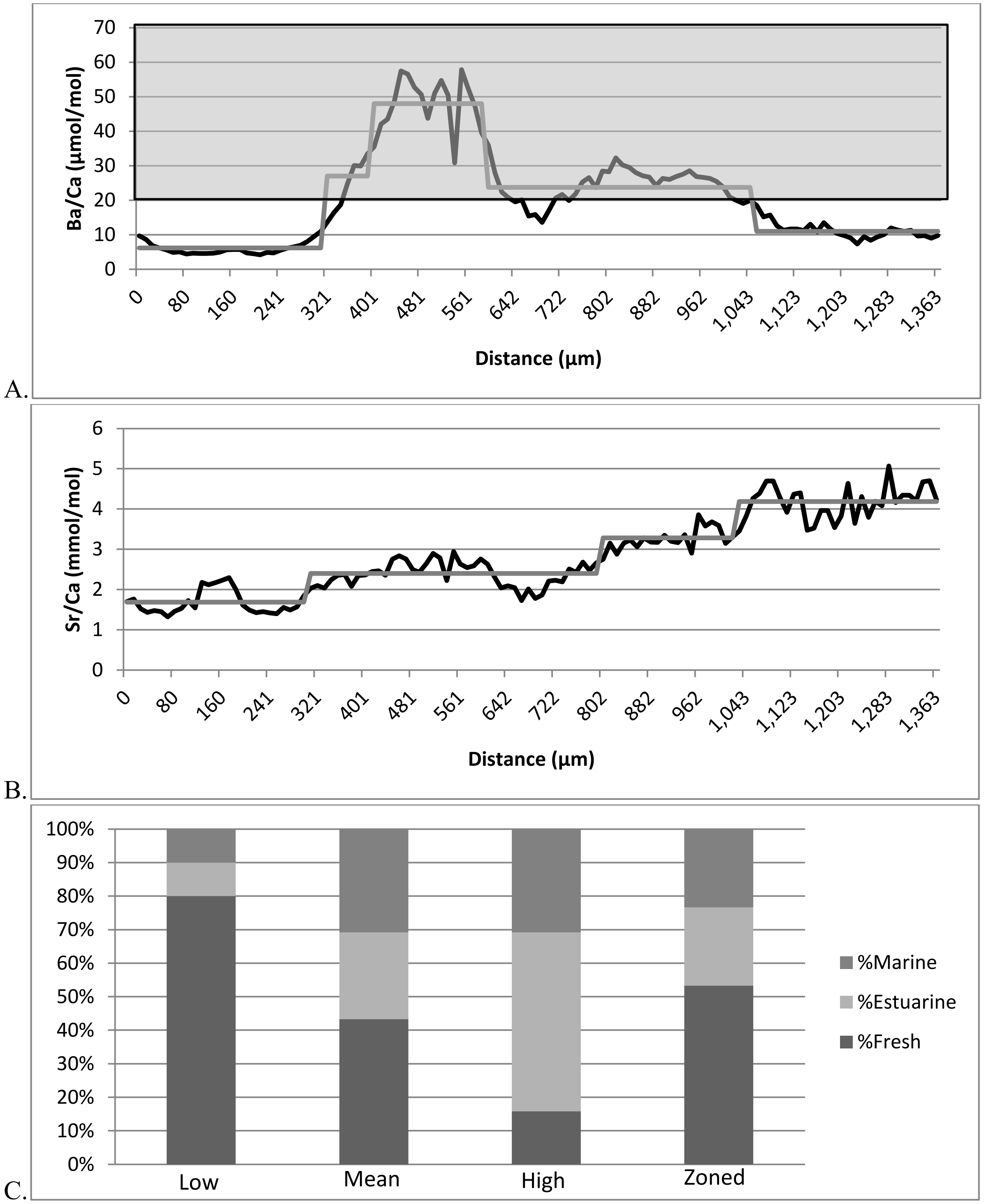


Figure AB.92. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 128. Figure 92.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

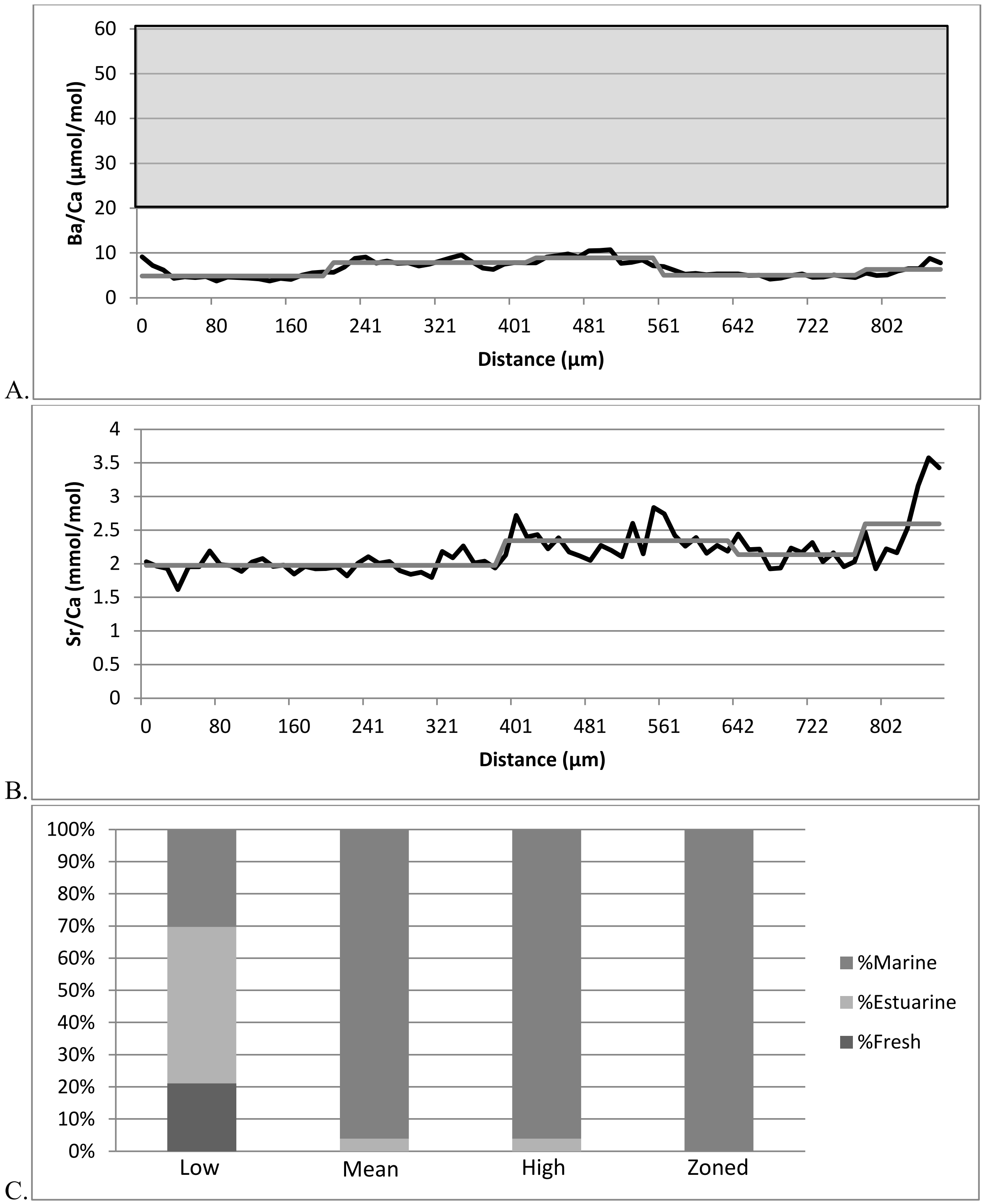
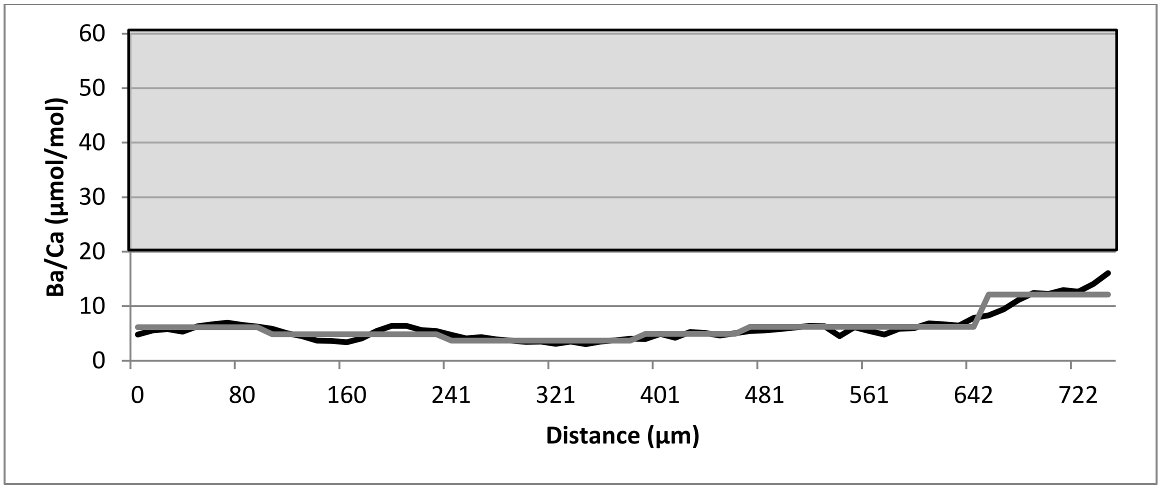
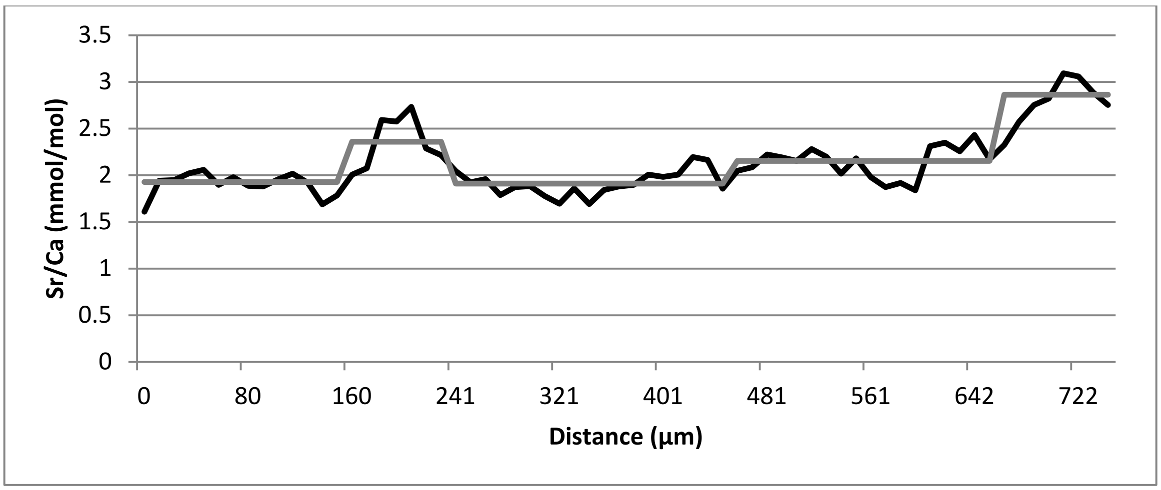


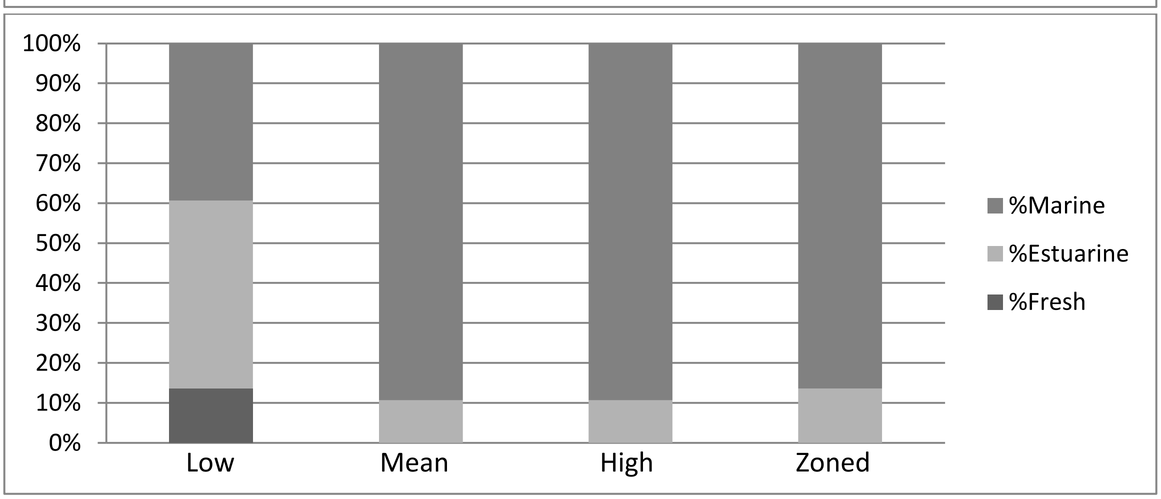
Figure AB.93. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 129. Figure 93.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

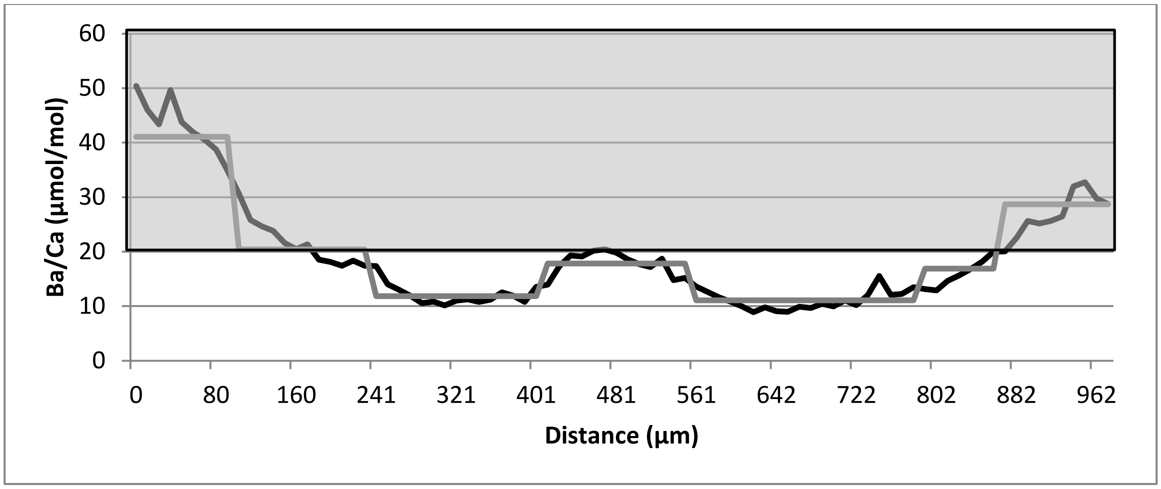


B.

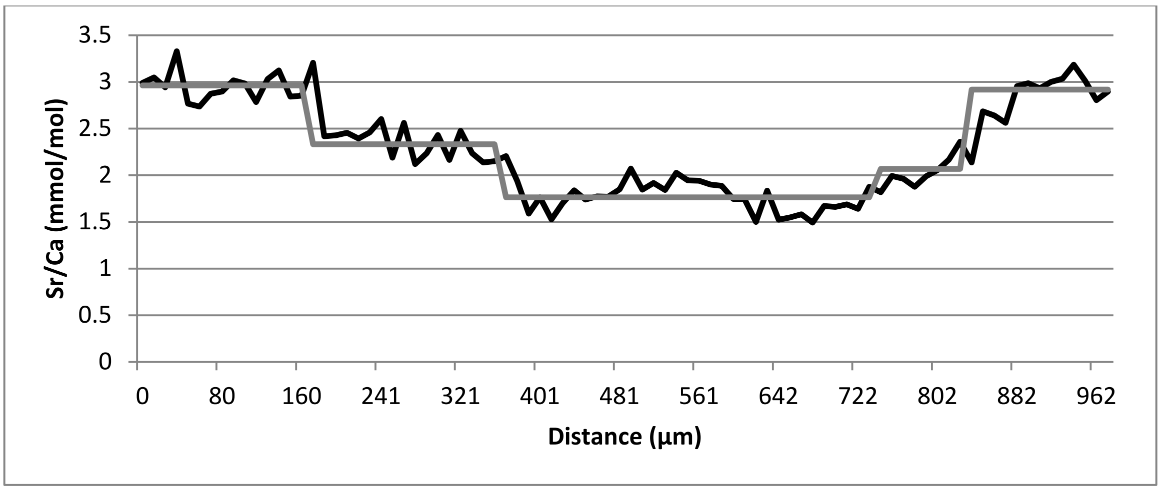


C.

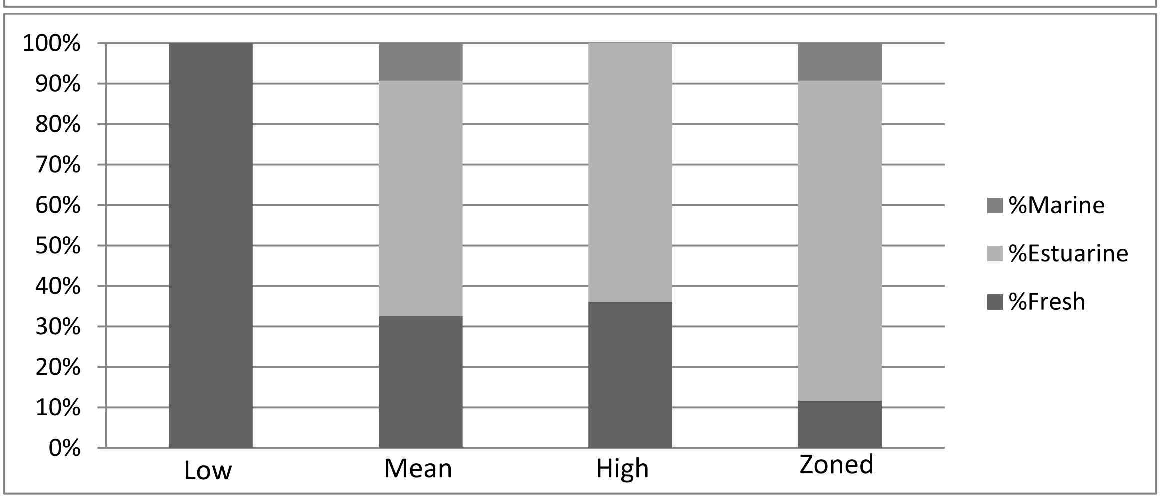
Figure AB.94. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 130. Figure 94.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

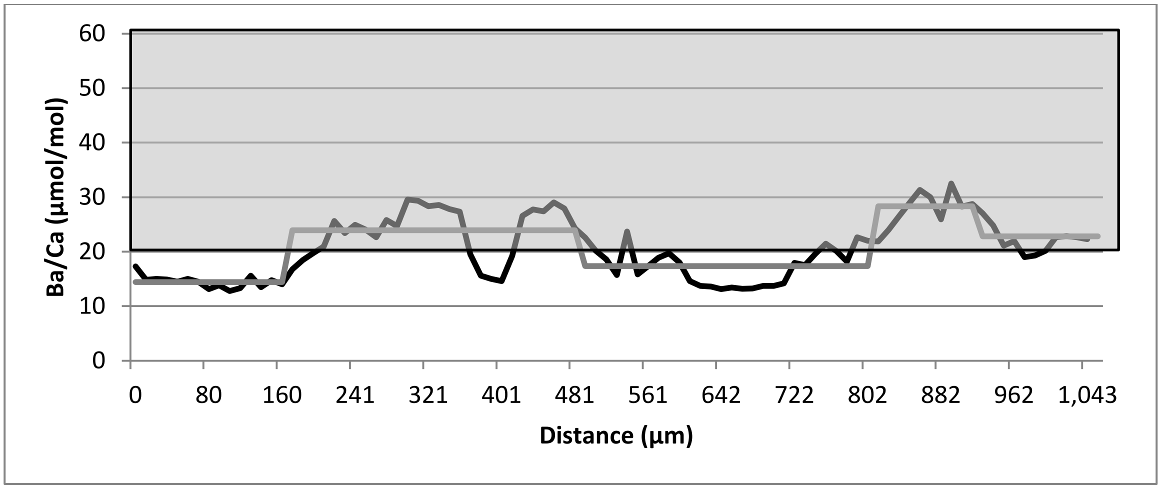


B.

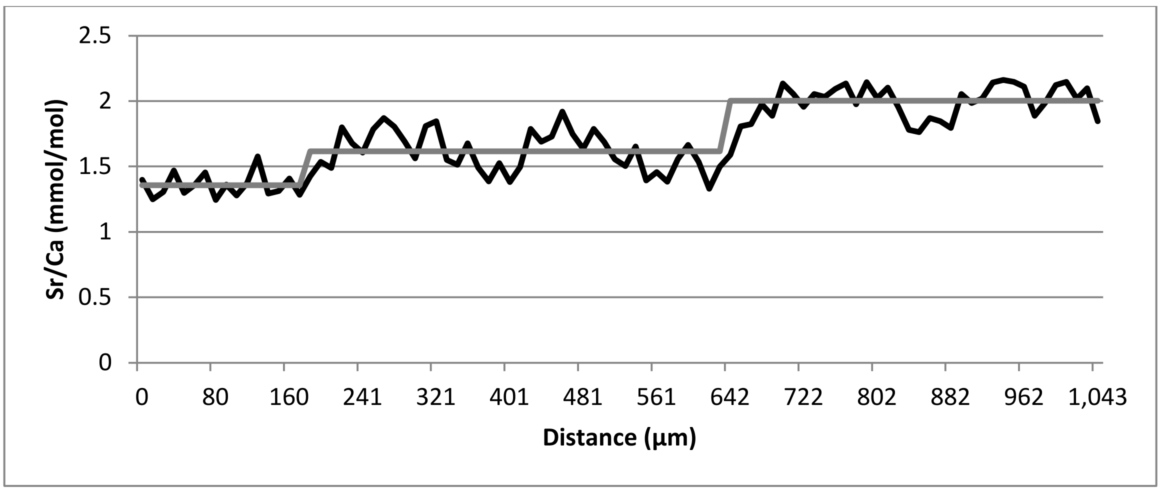


C.

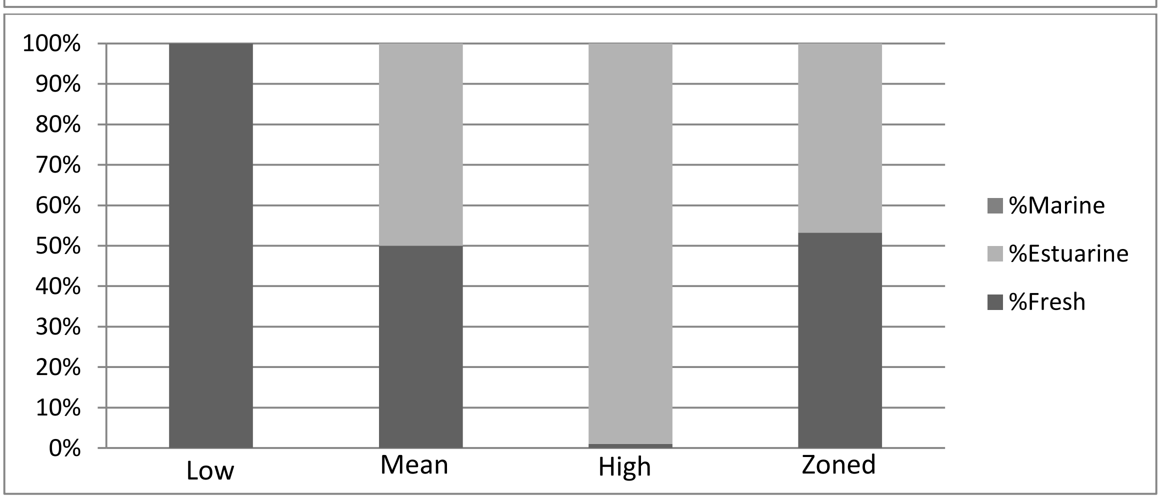
Figure AB.95. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 131. Figure 95.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

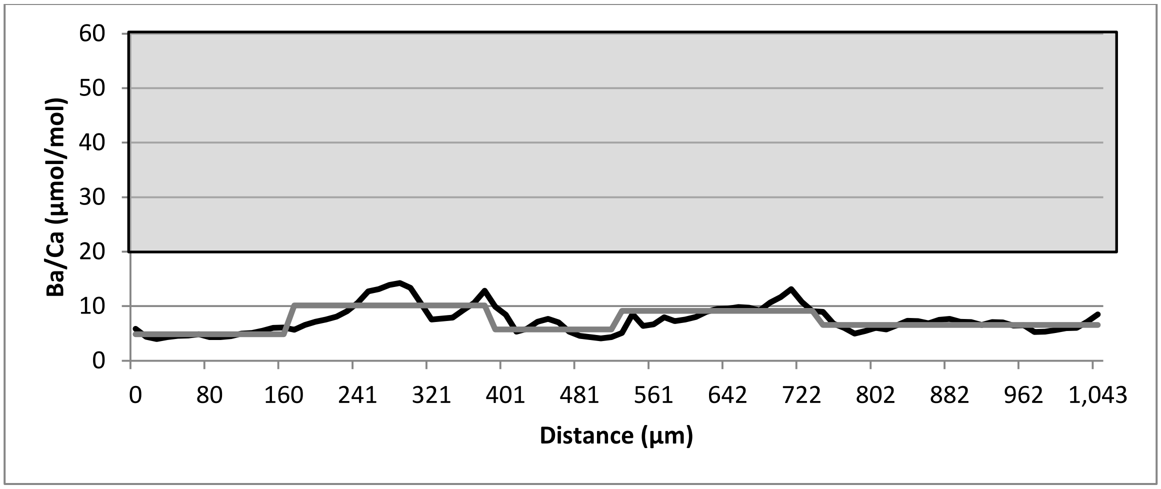


B.

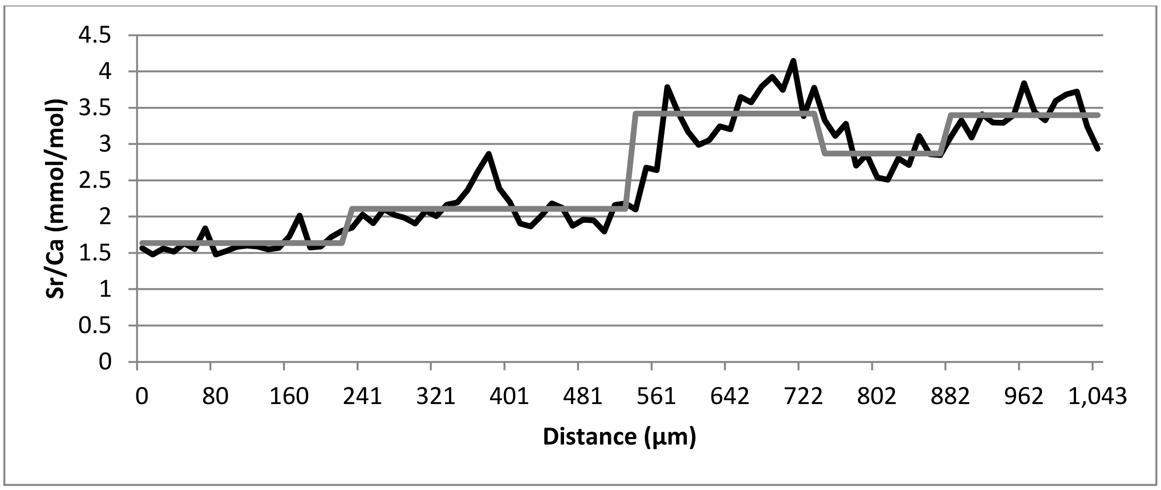


C.

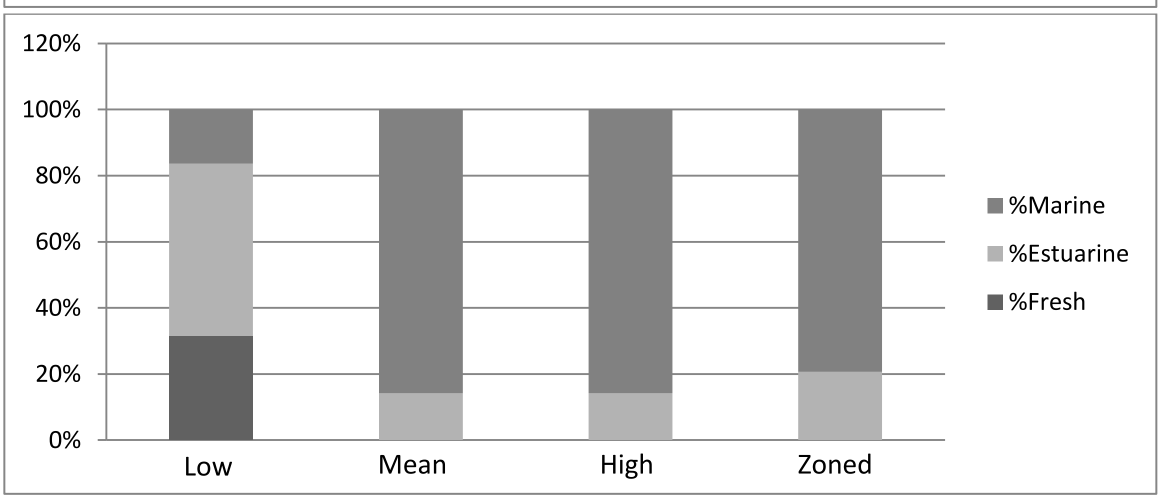
Figure AB.96. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 132. Figure 96.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

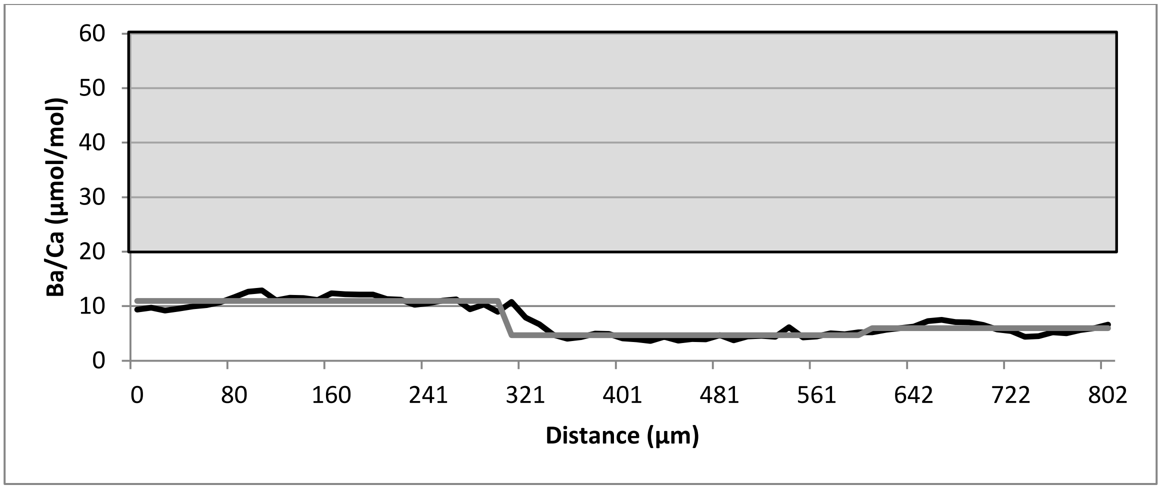


B.

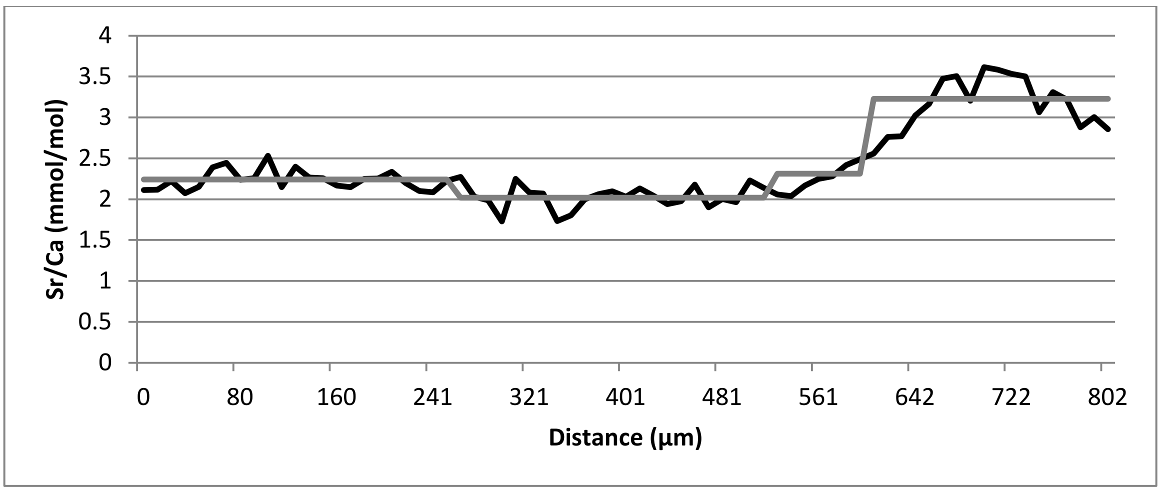


C.

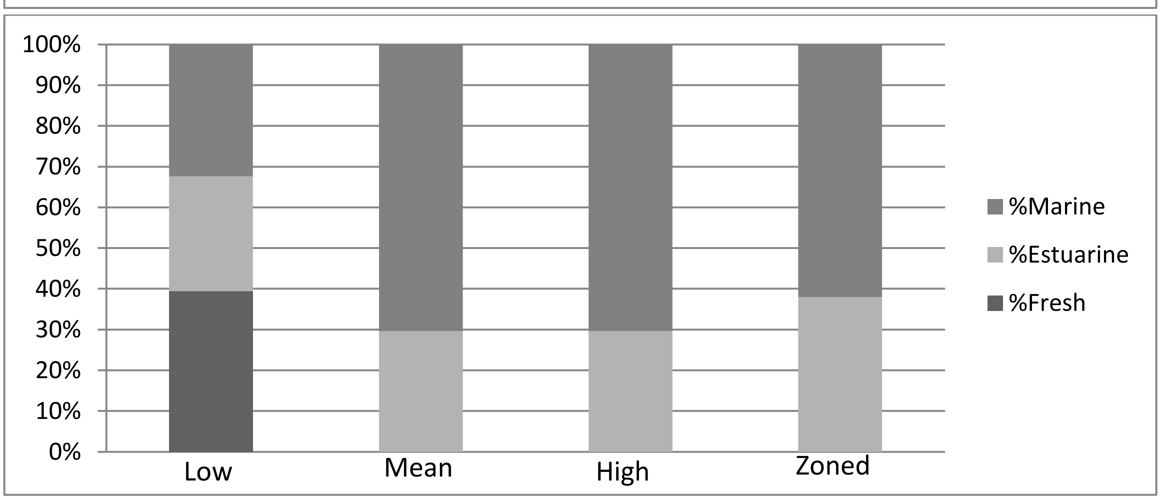
Figure AB.97. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 133. Figure 97.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.98. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 134. Figure 98.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

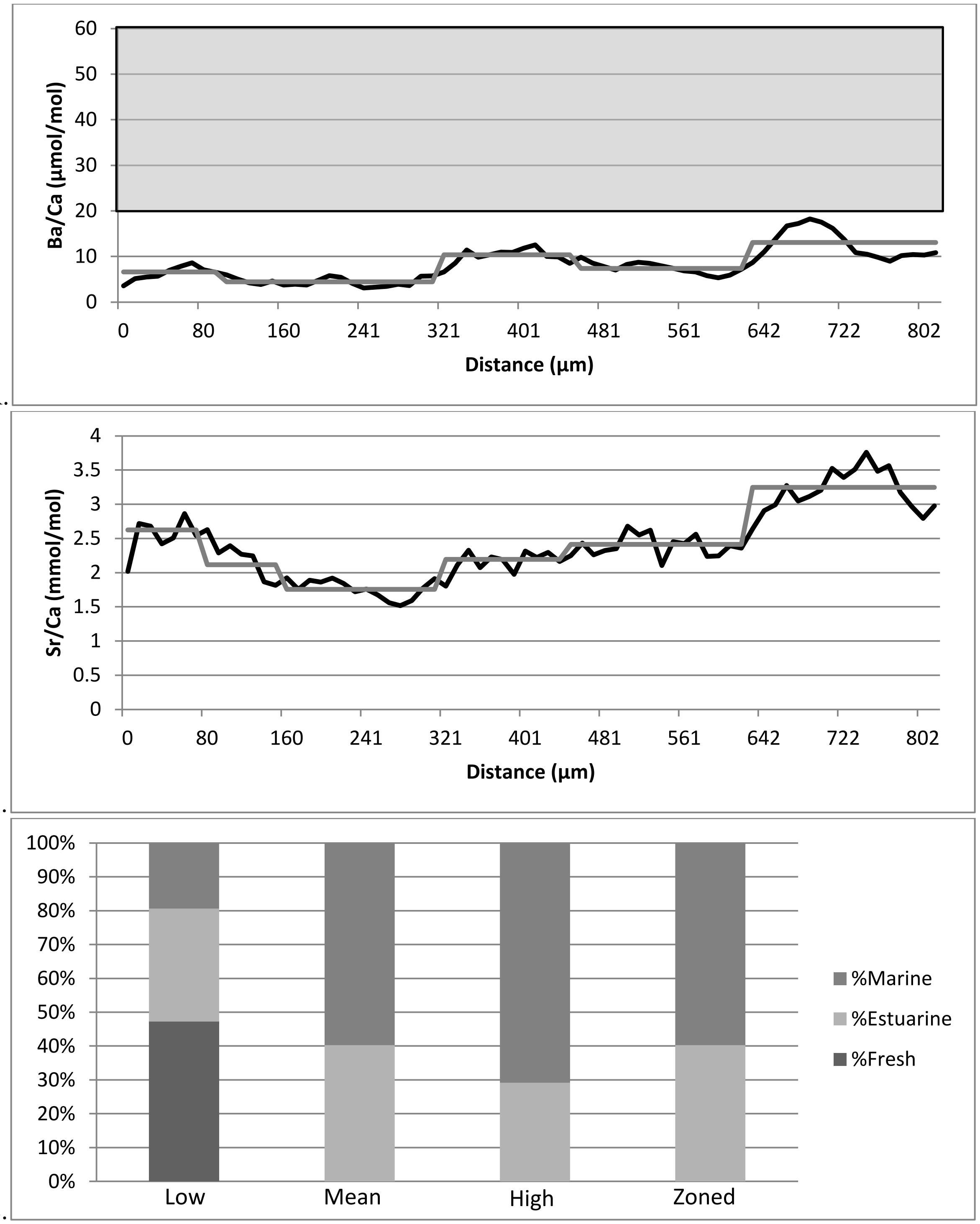
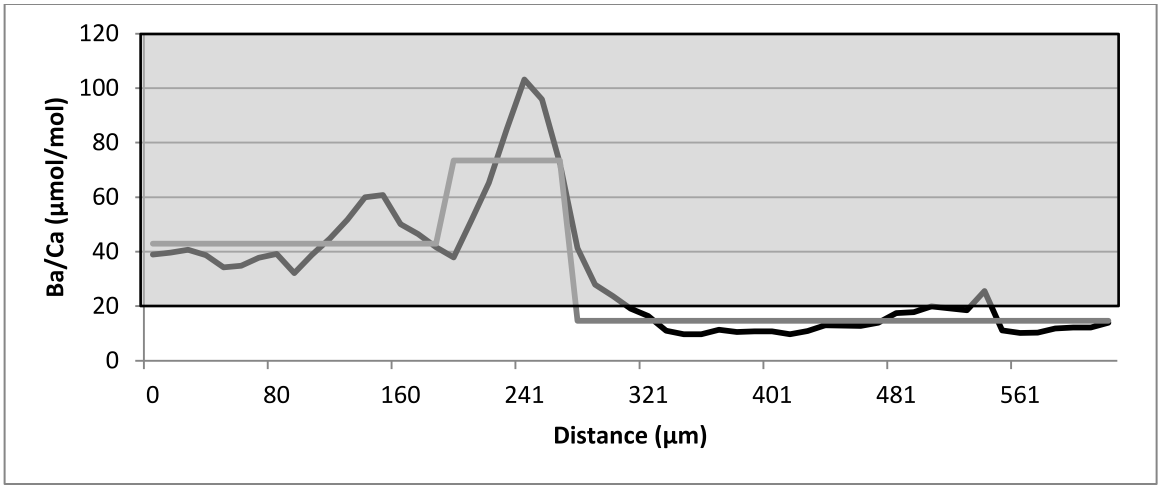
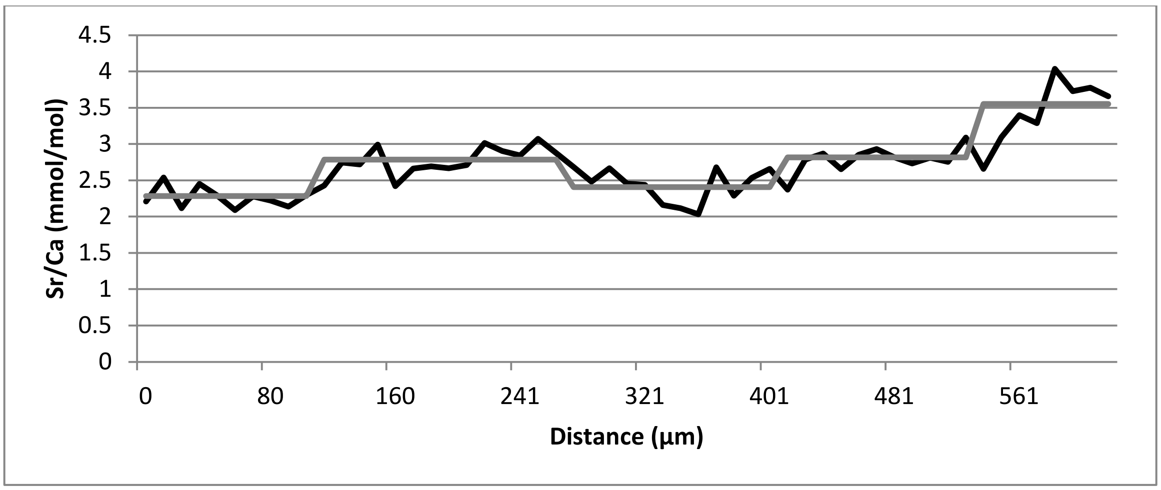
A.
B.
C.


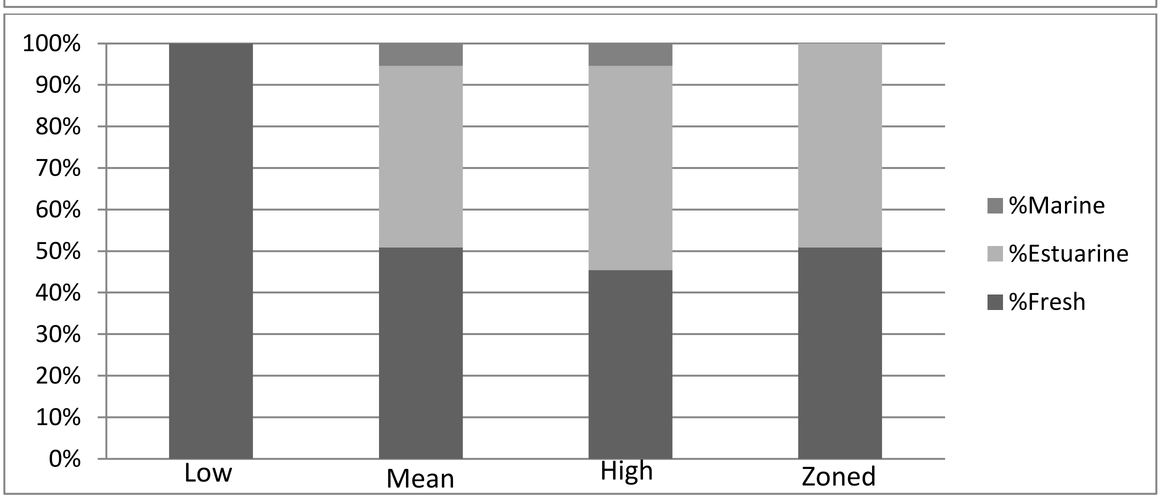
Figure AB.99. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 135. Figure 99.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

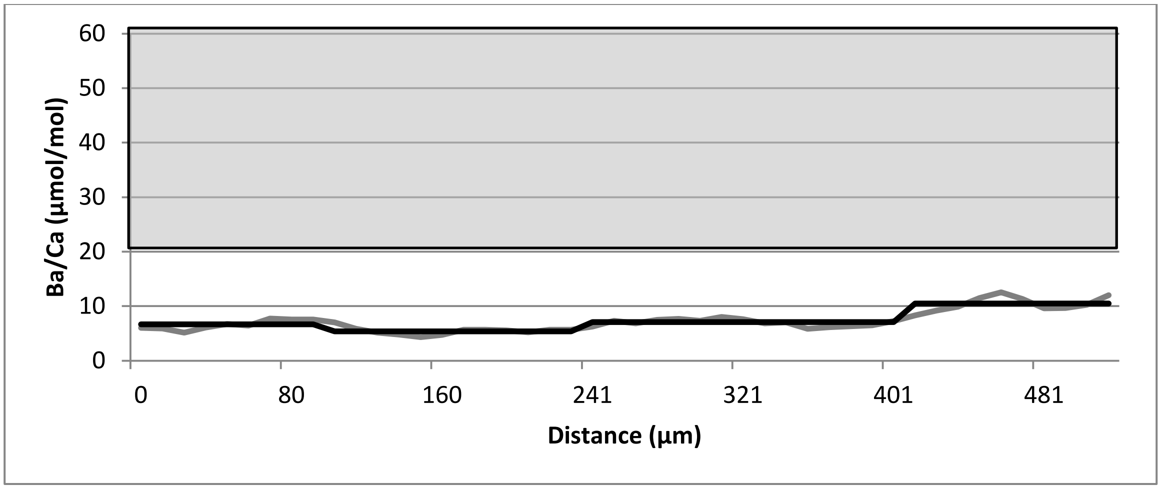


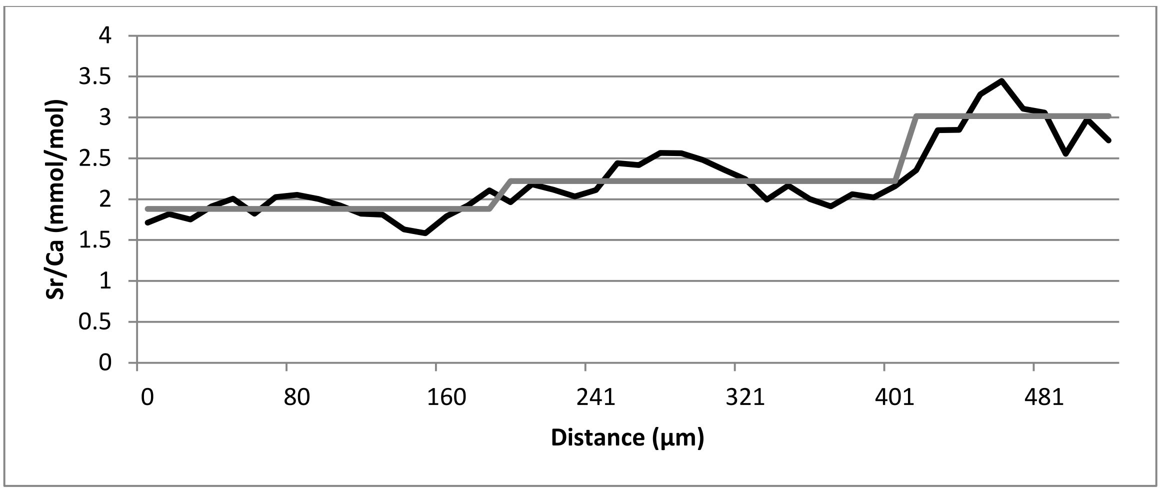
B.

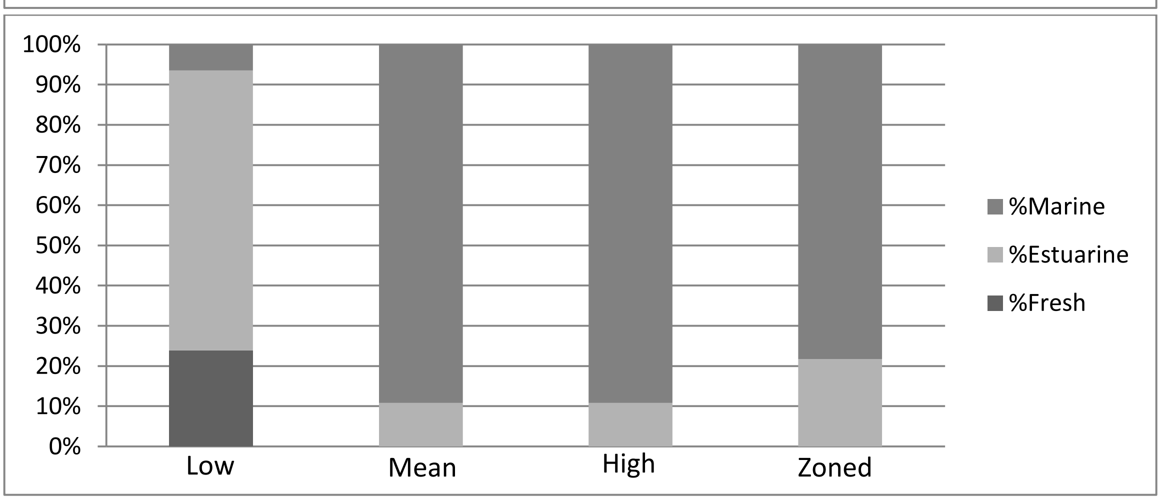


C.

Figure AB.100. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 136. Figure 100.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

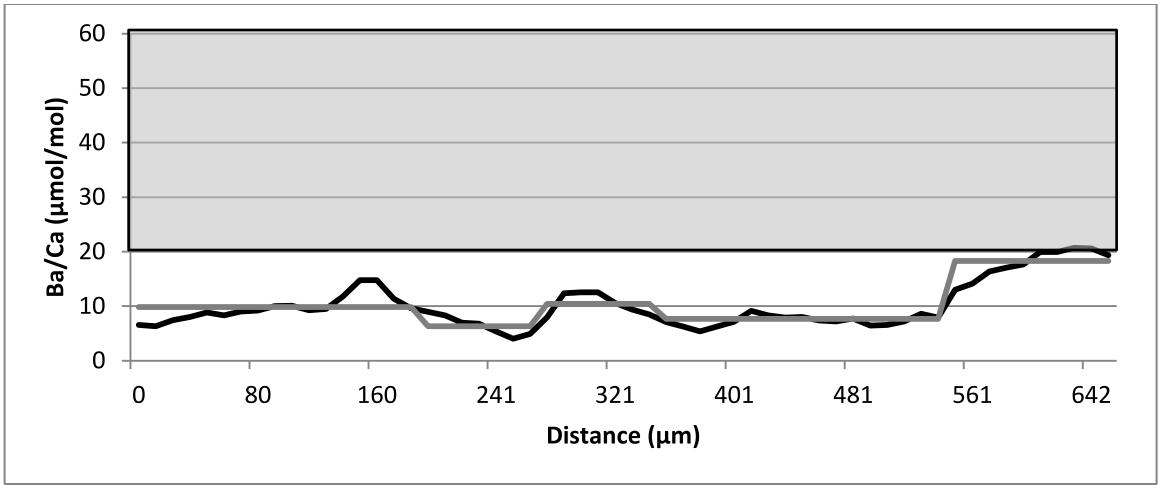


A.


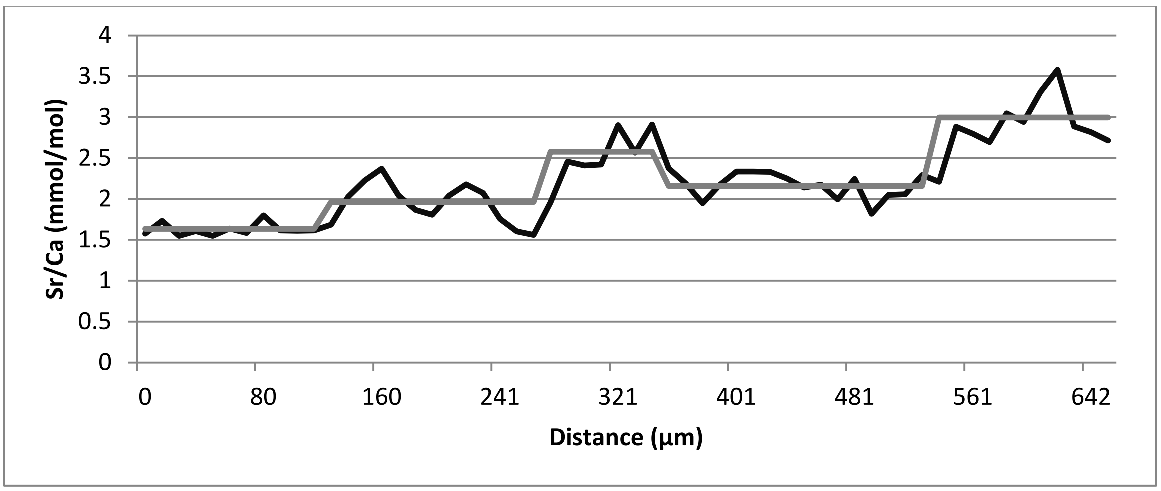
B.


C.

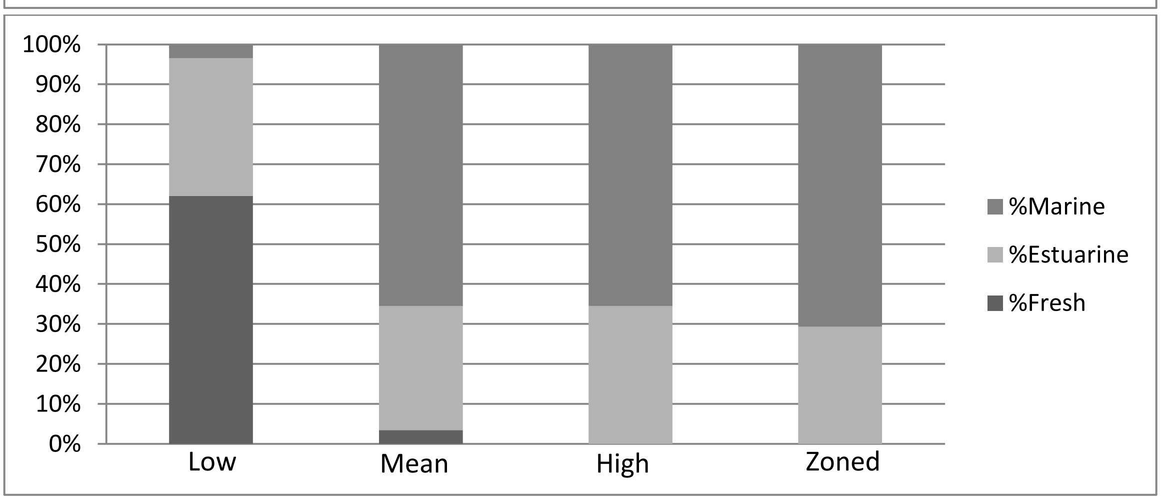
Figure AB.101. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 137. Figure 101.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

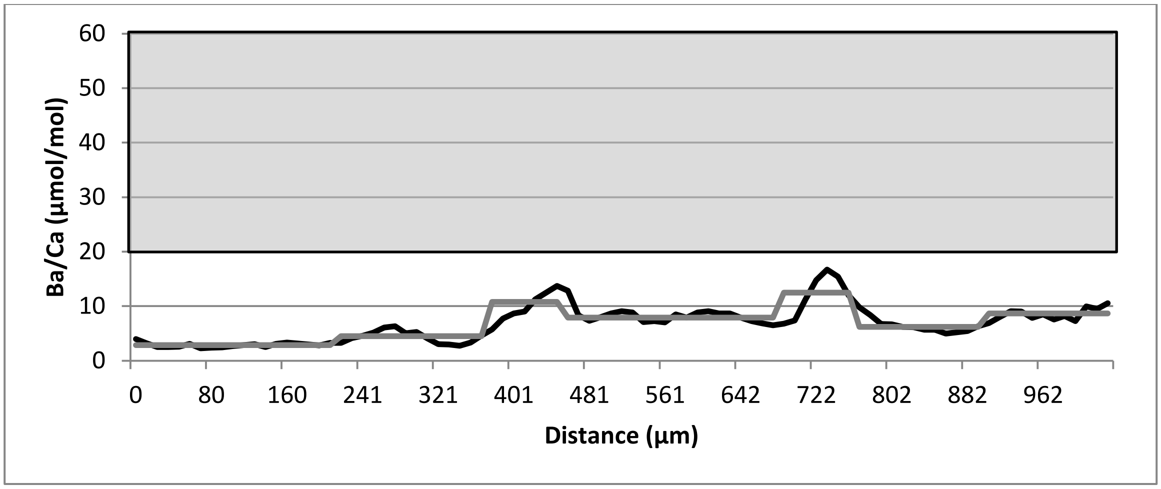


B.

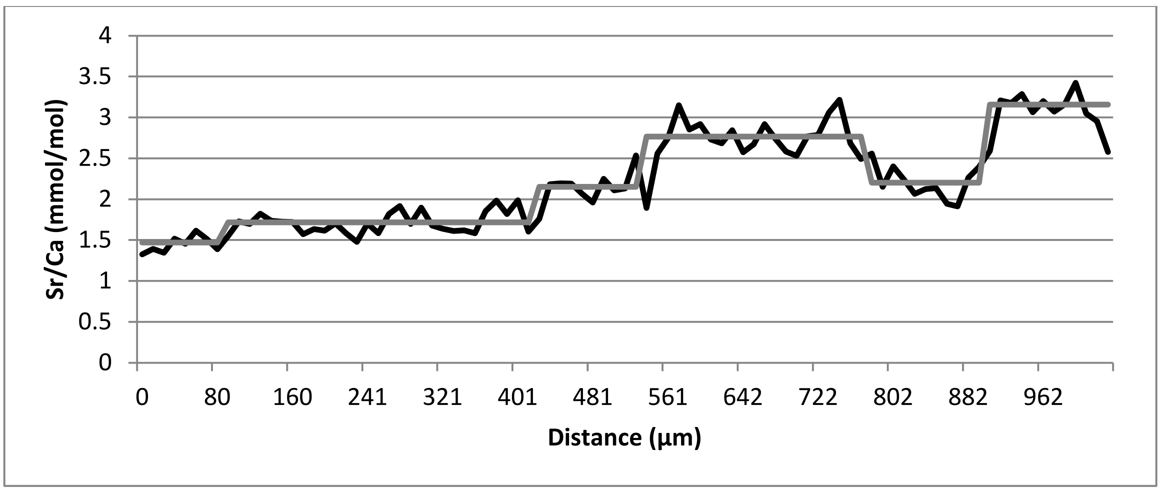


C.

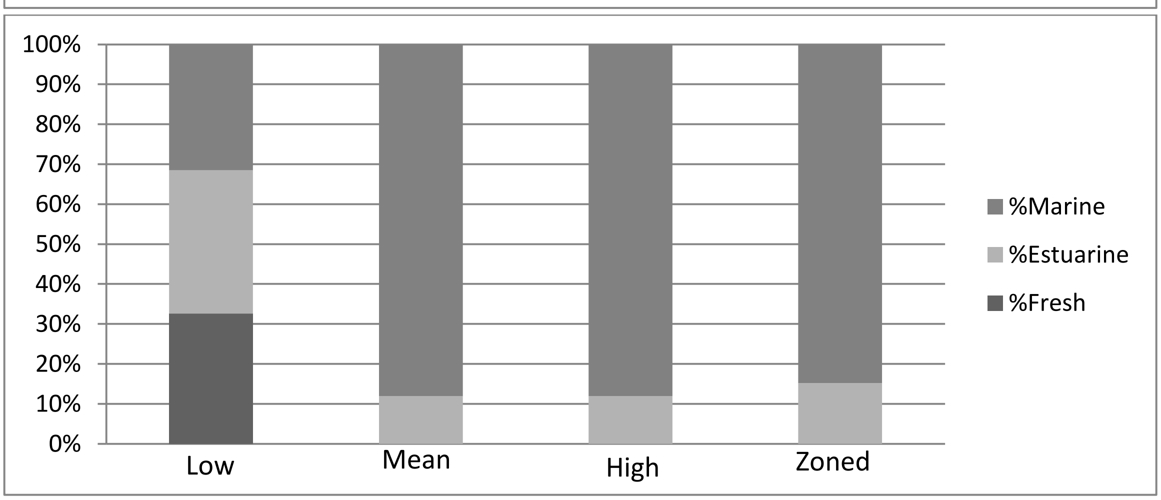
Figure AB.102. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 138. Figure 102.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.103. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 139. Figure 103.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

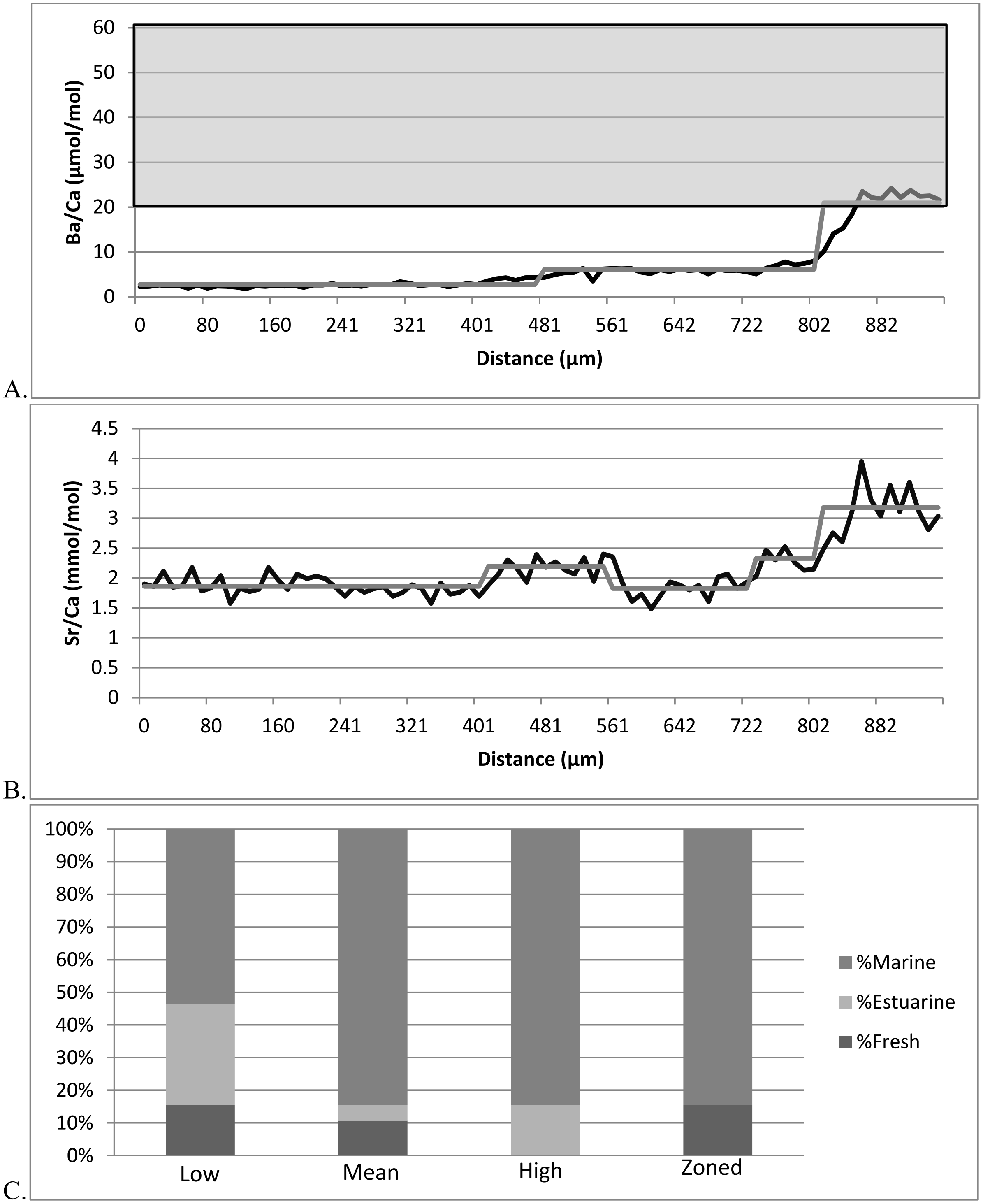
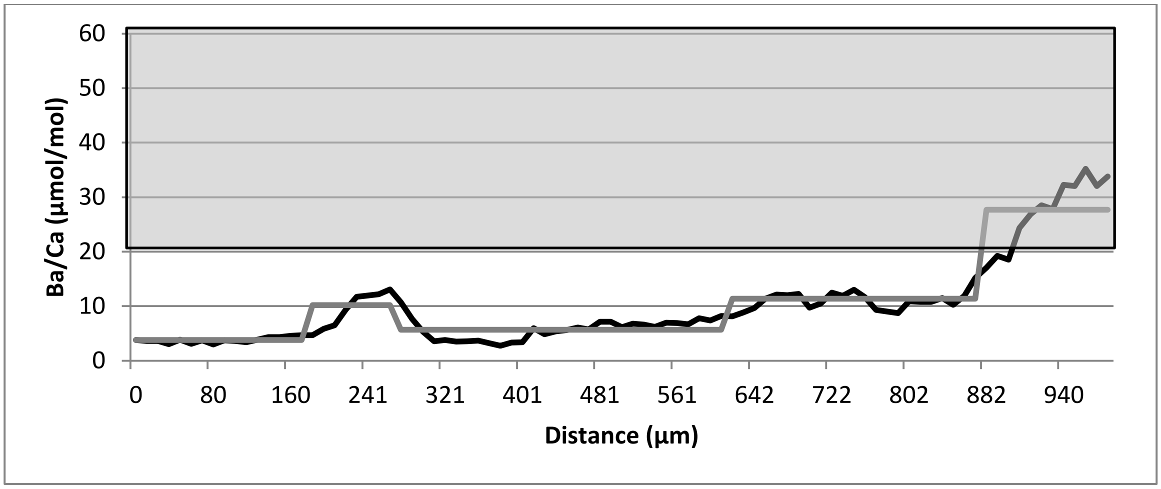
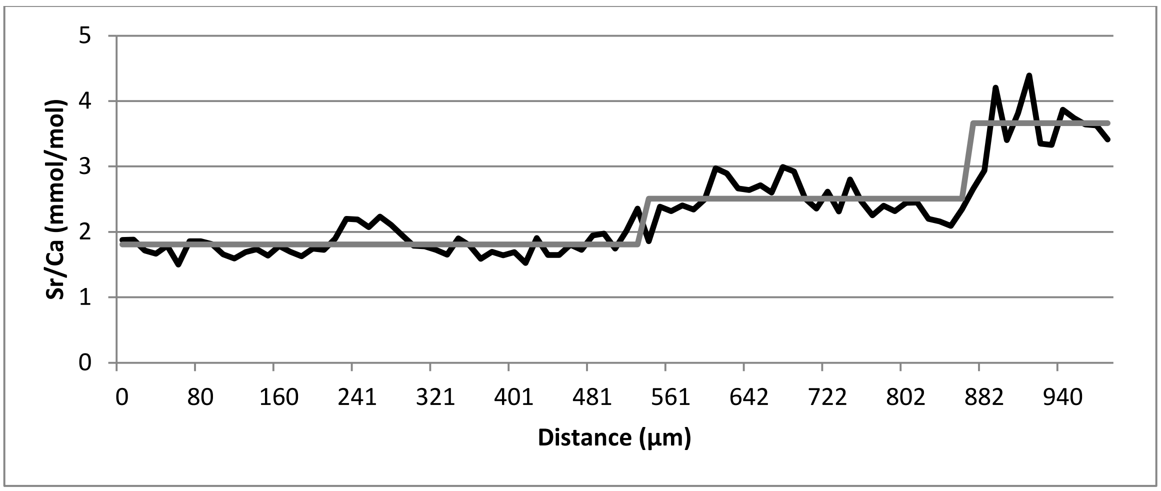


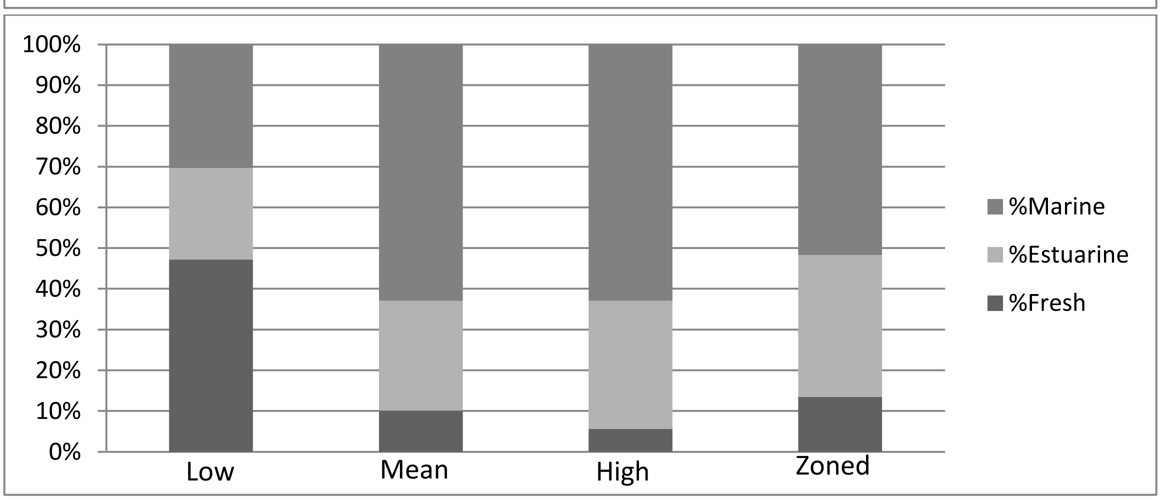
Figure AB.104. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 140. Figure 104.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

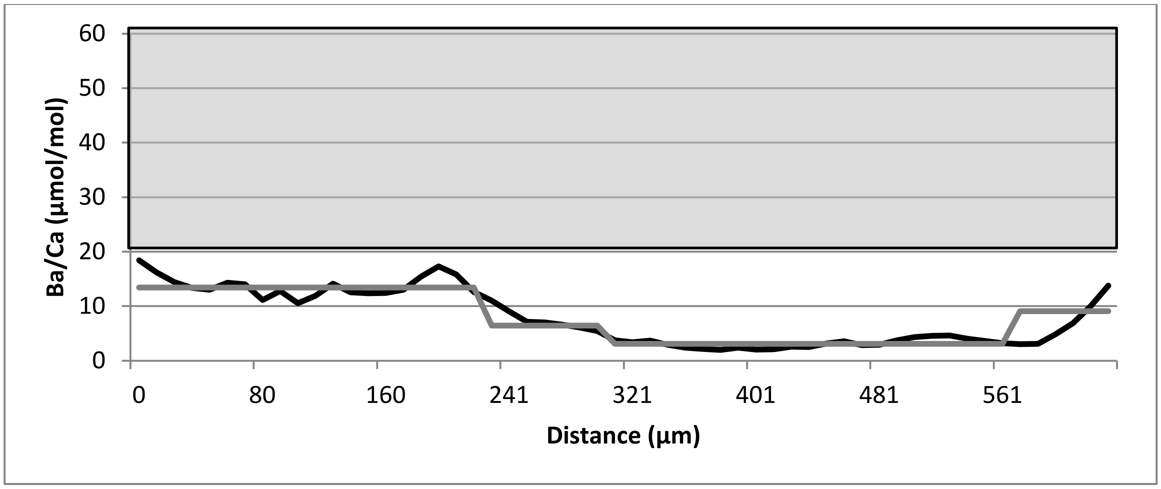


B.

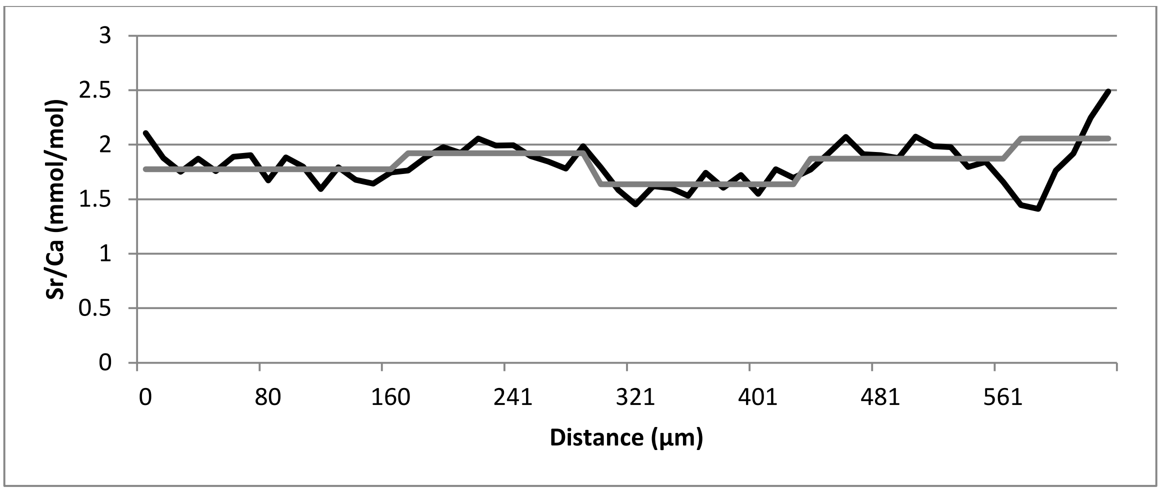


C.

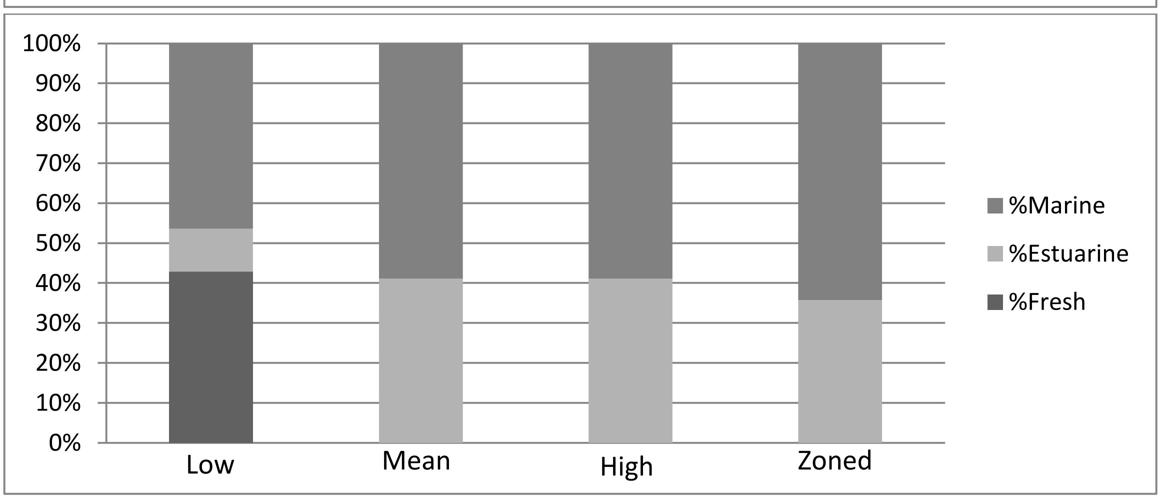
Figure AB.105. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 141. Figure 105.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

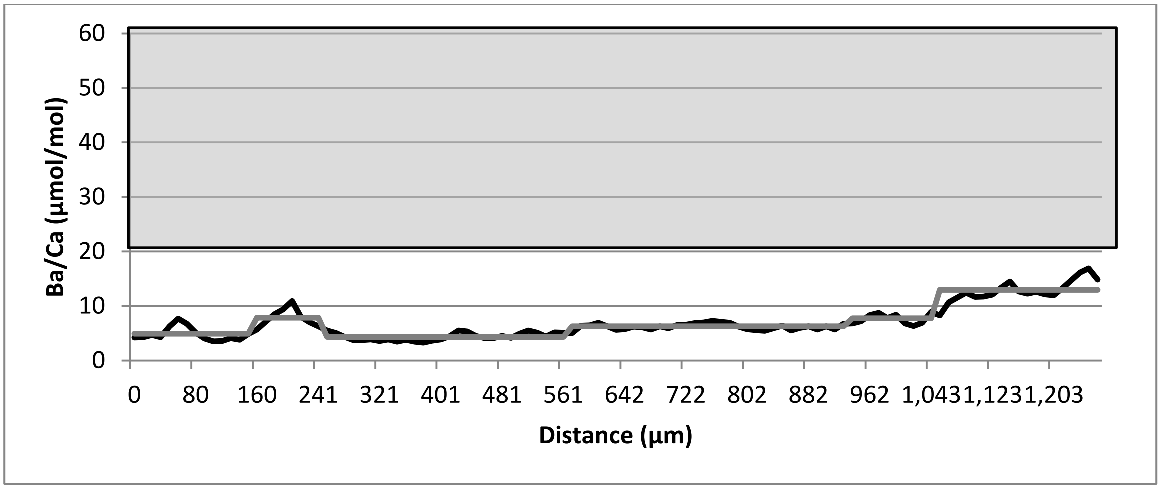


B.

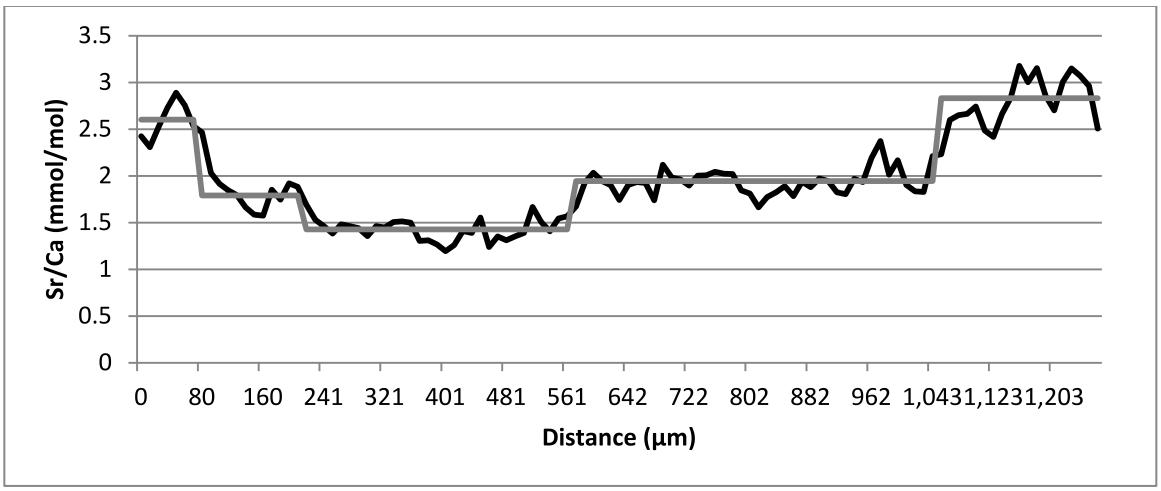


C.

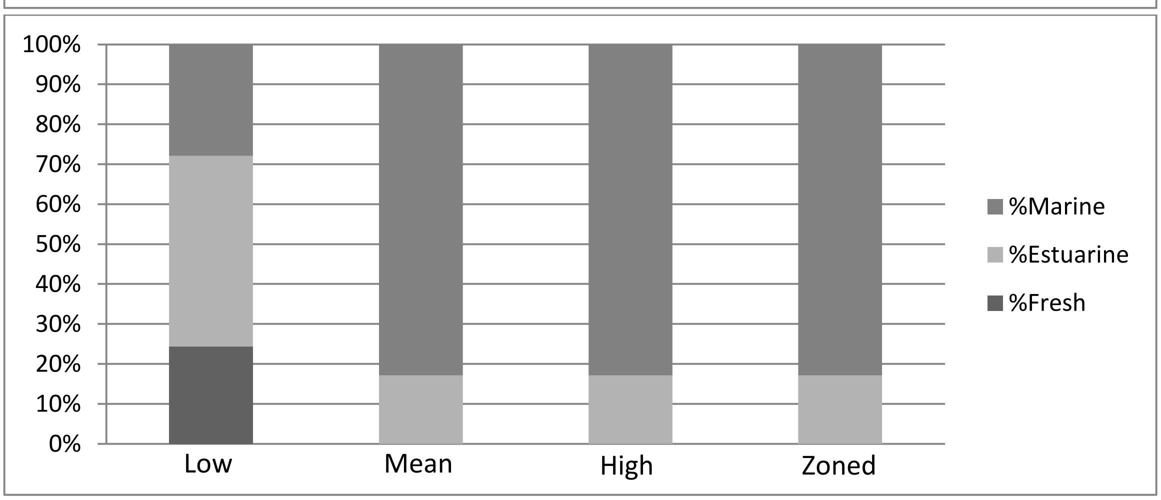
Figure AB.106. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 142. Figure 106.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.107. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 143. Figure 107.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

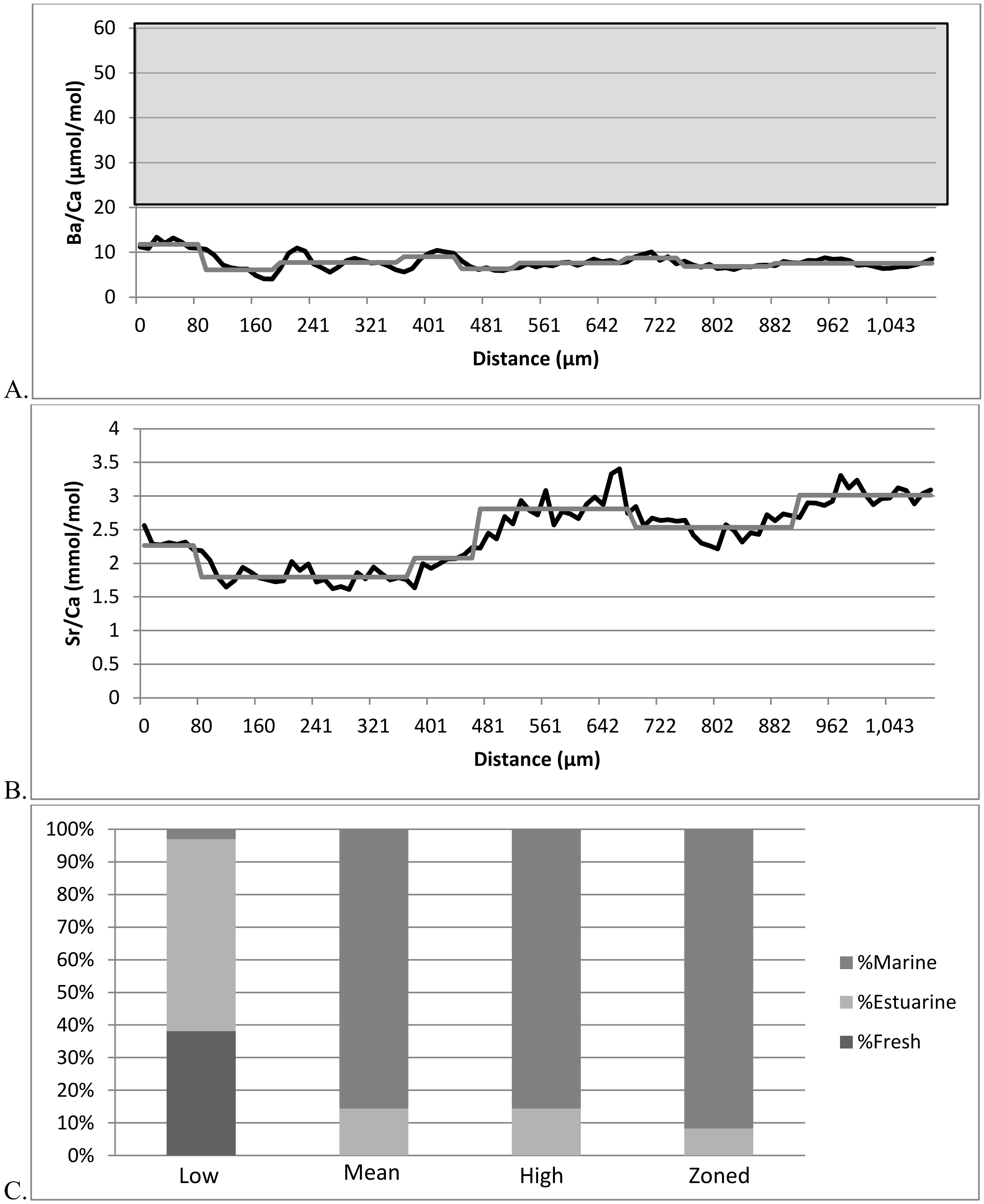
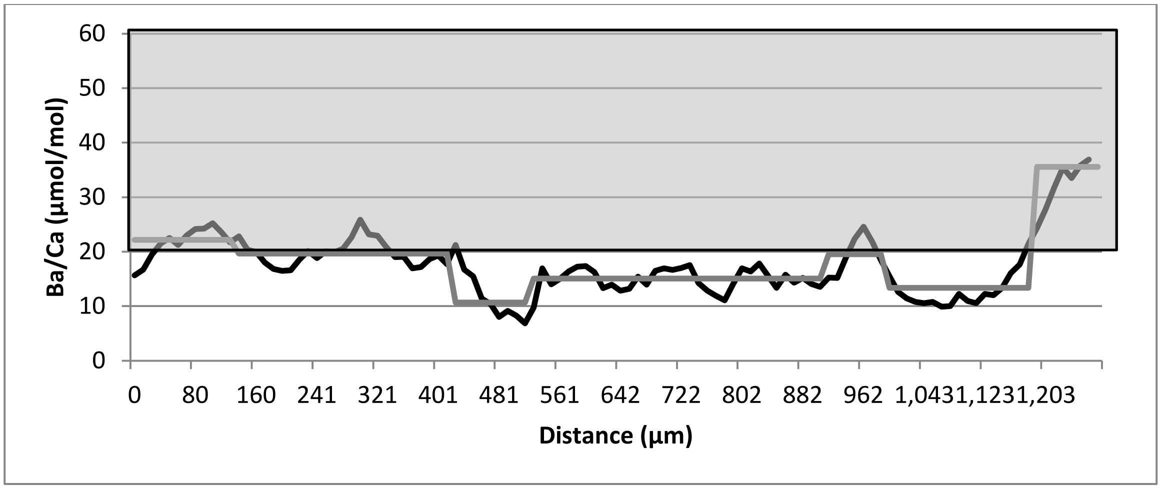
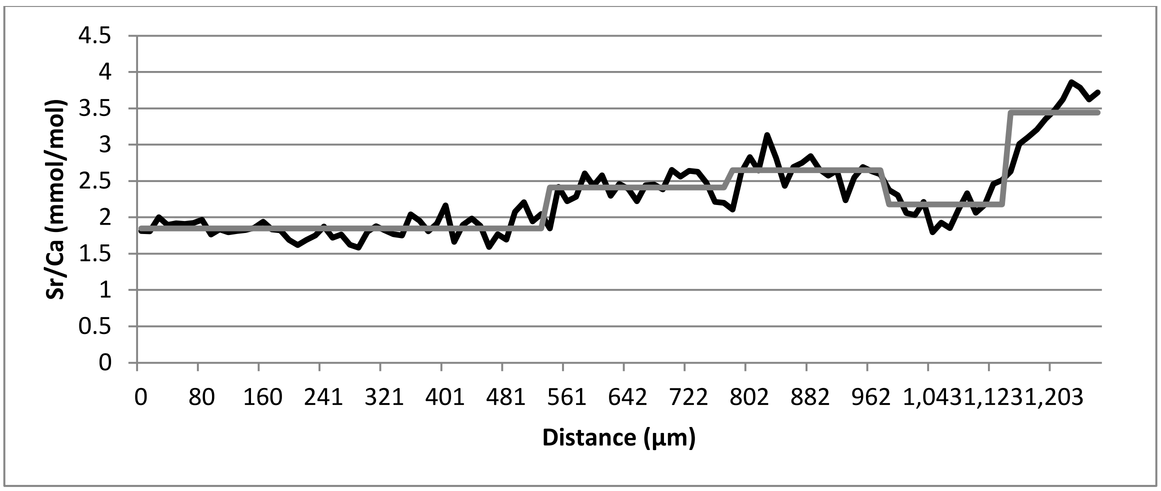


Figure AB.108. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 145. Figure 108.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


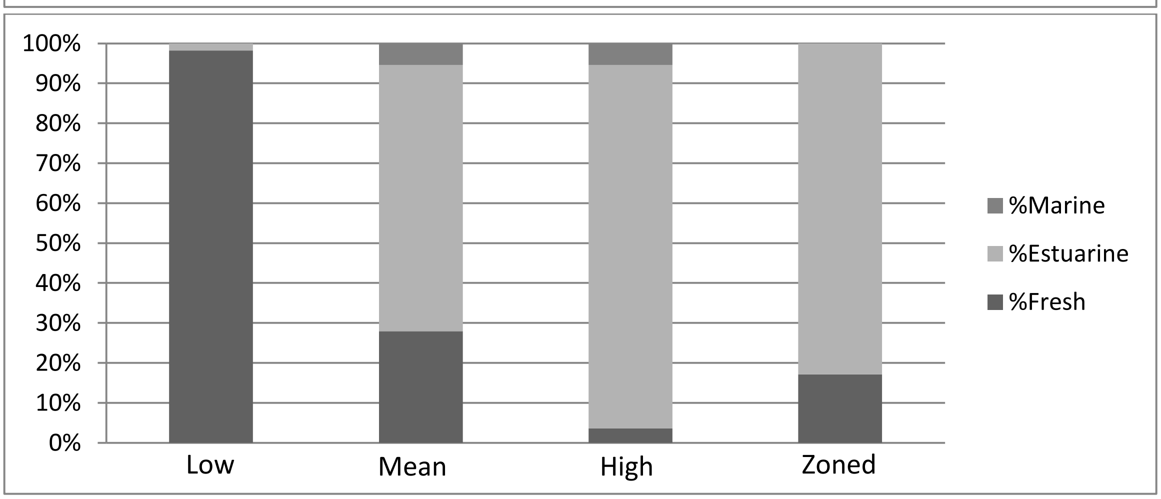
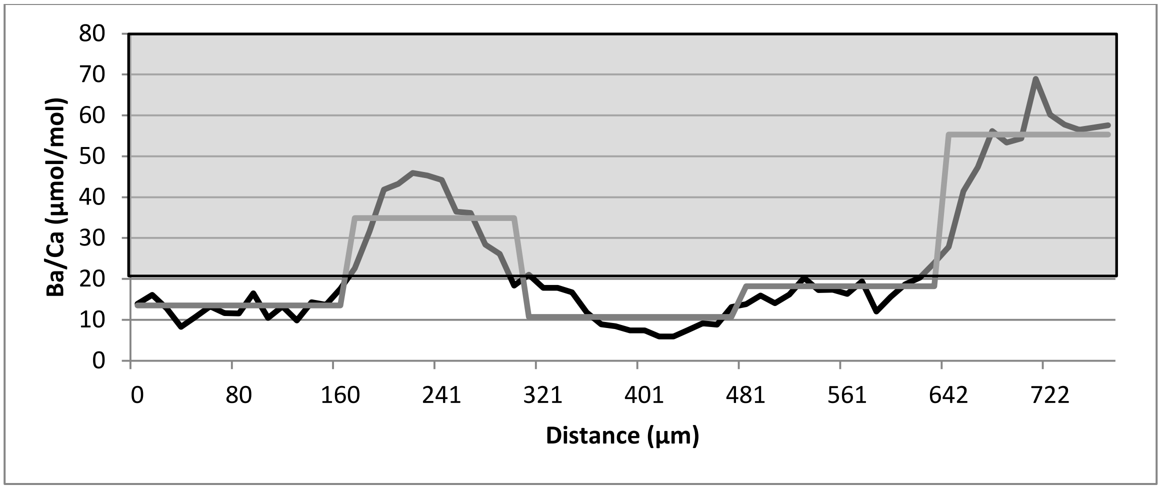
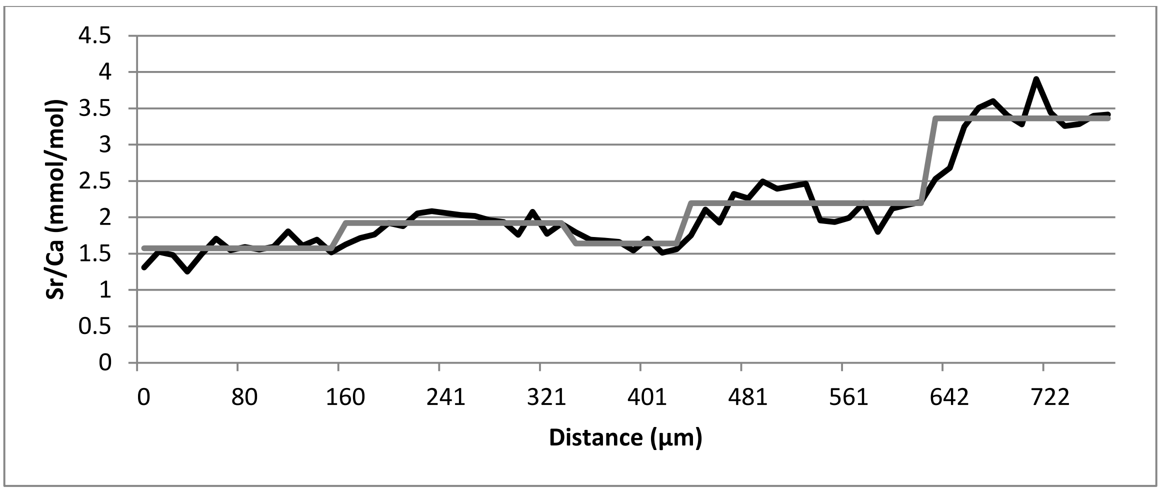
C.


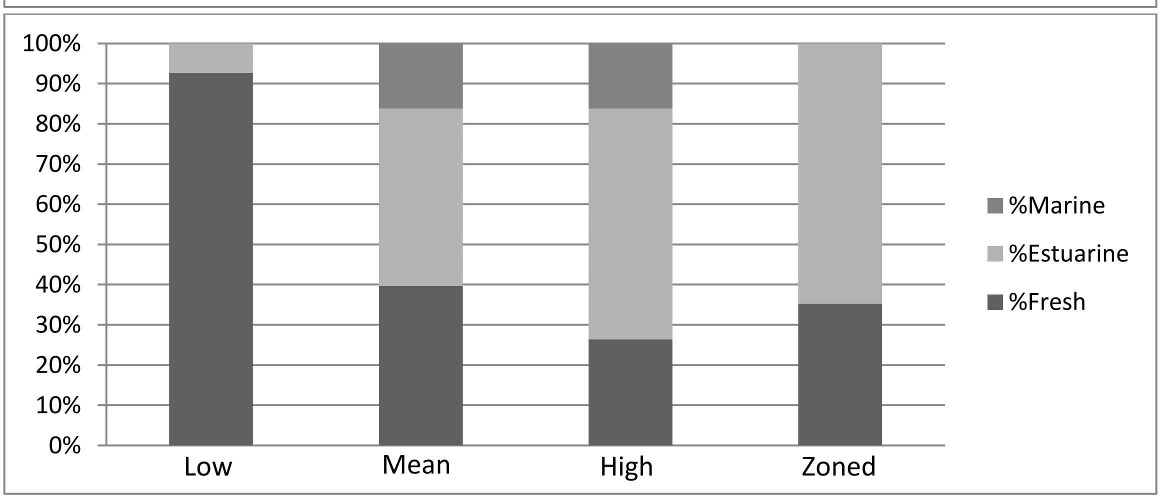
Figure AB.109. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 146. Figure 109.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

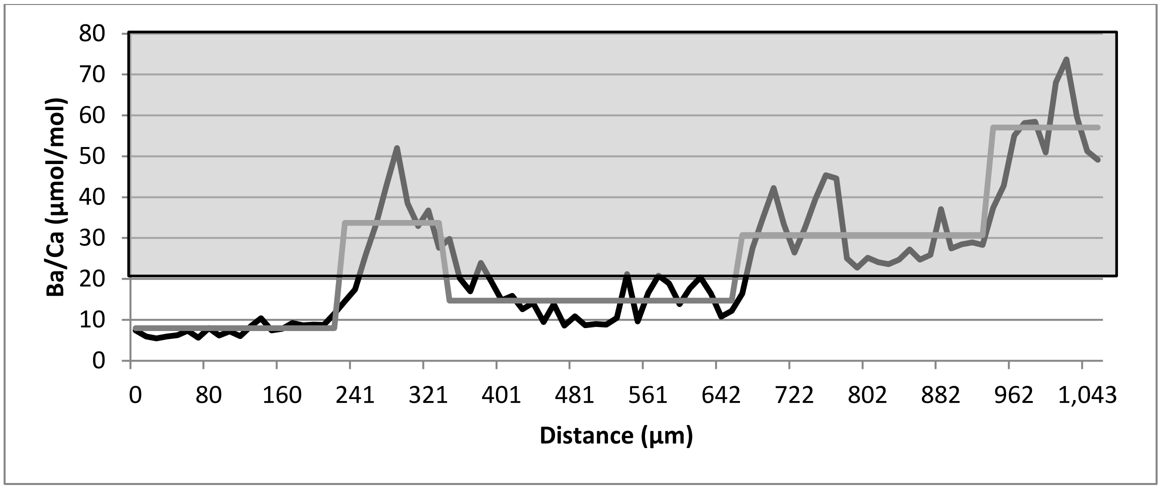


B.

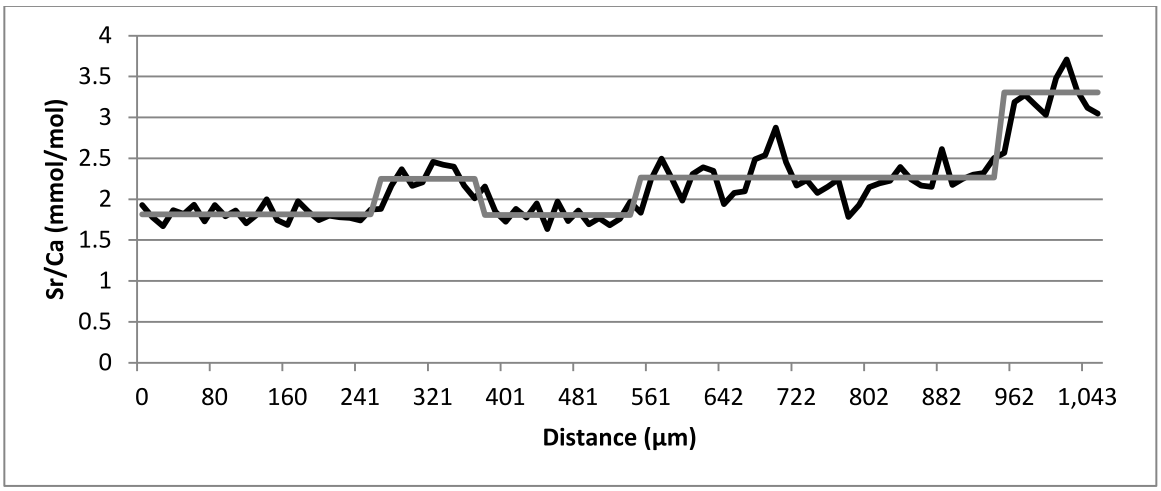


C.

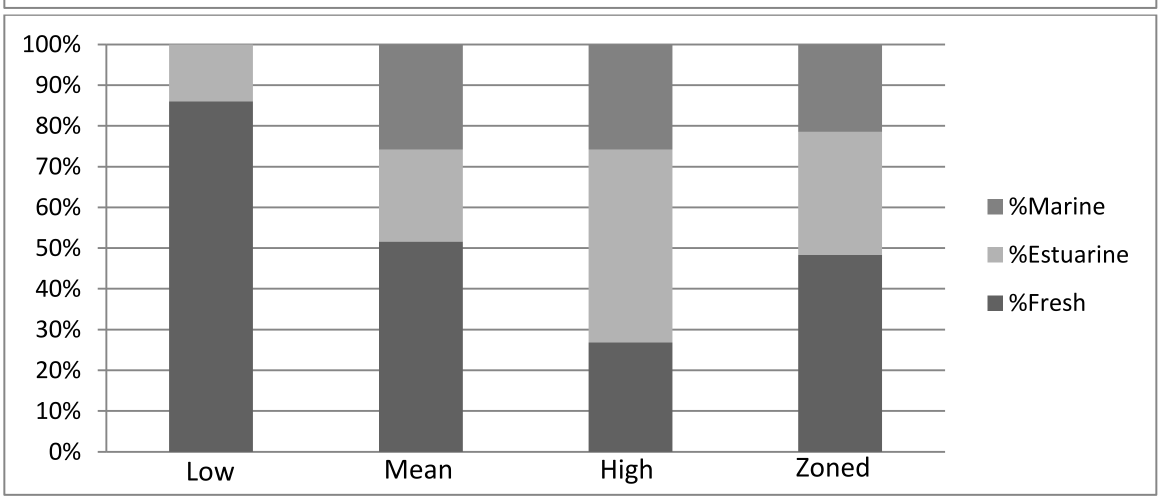
Figure AB.110. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 147. Figure 110.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

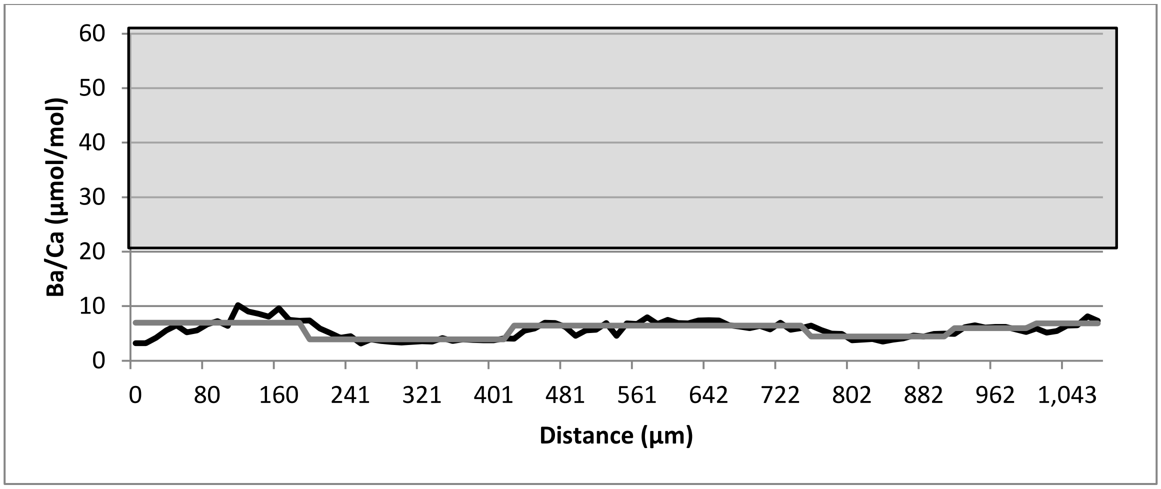


B.

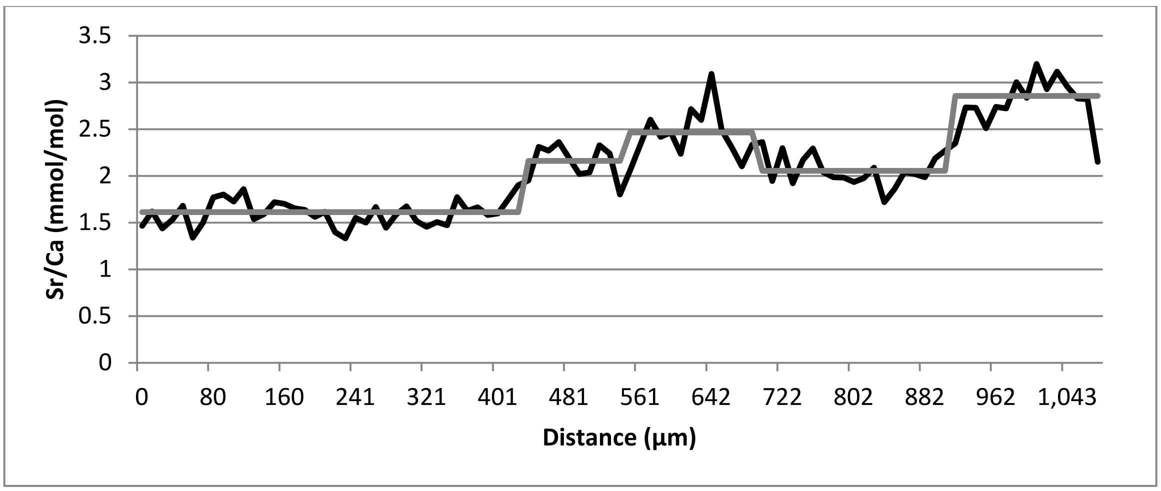


C.

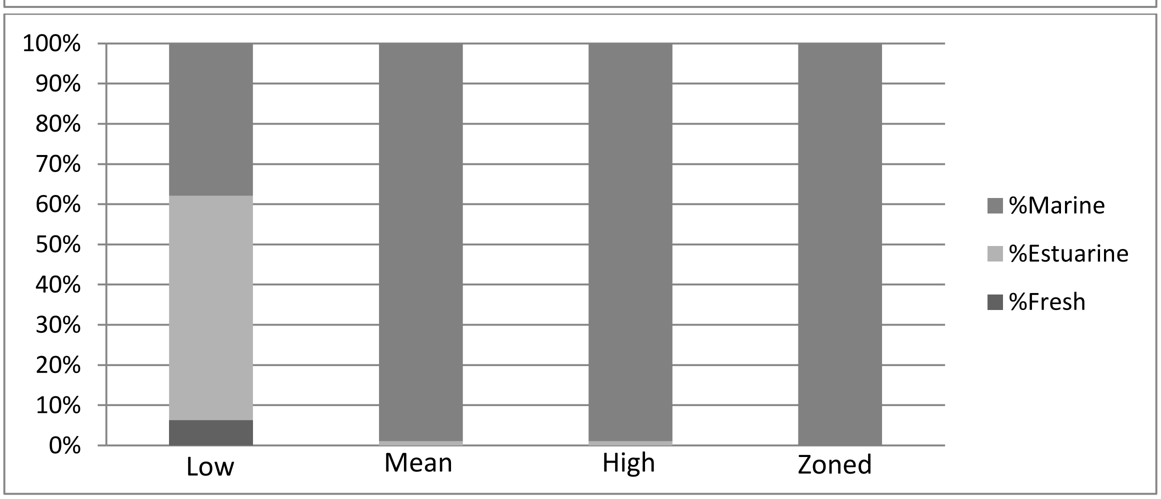
Figure AB.111. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 148. Figure 111.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.112. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 149. Figure 112.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

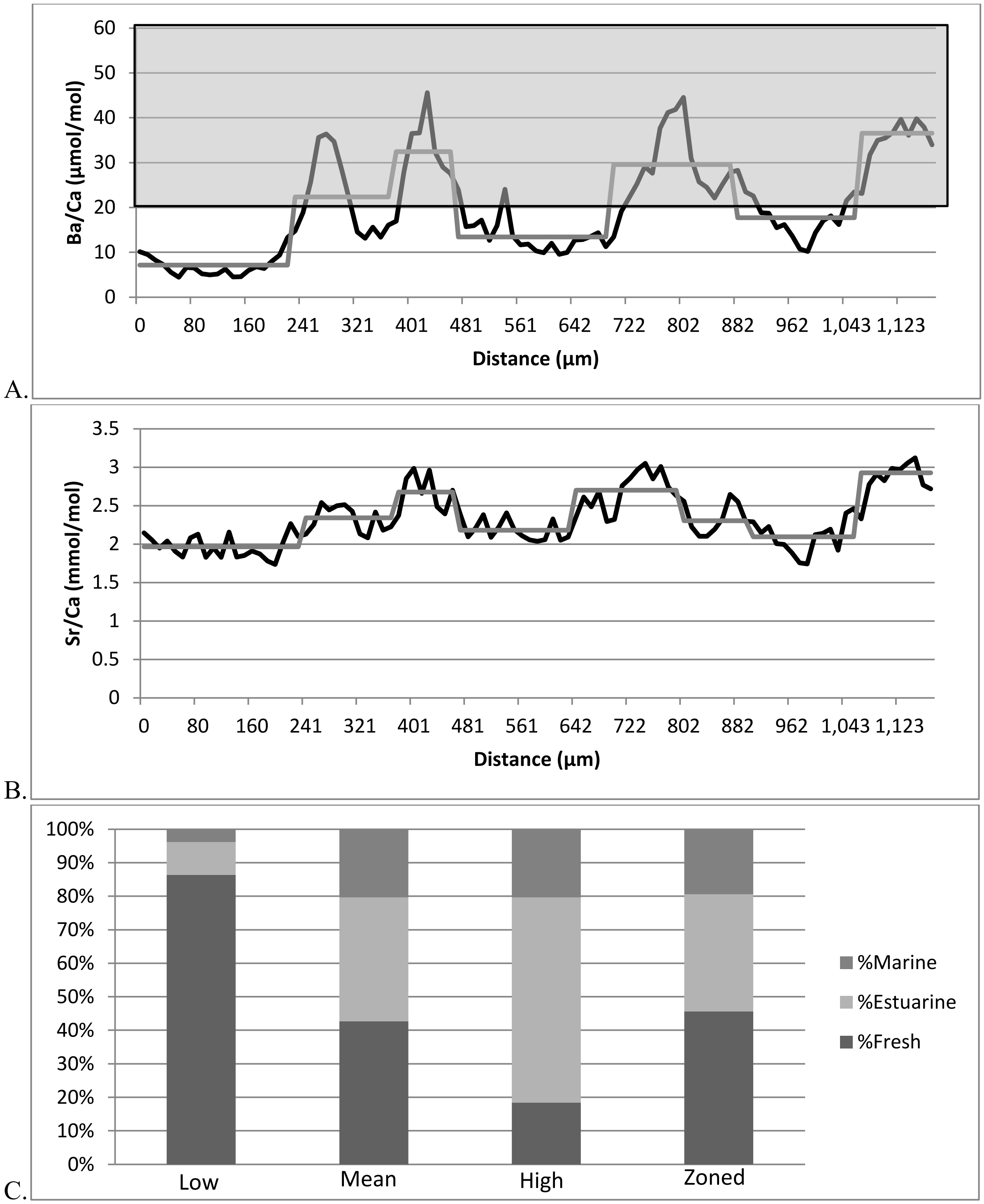
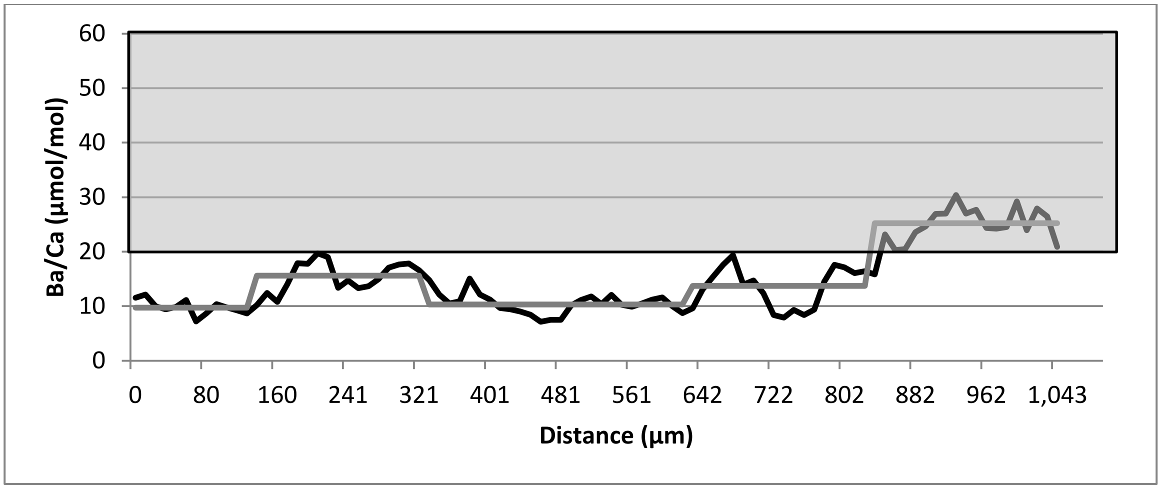
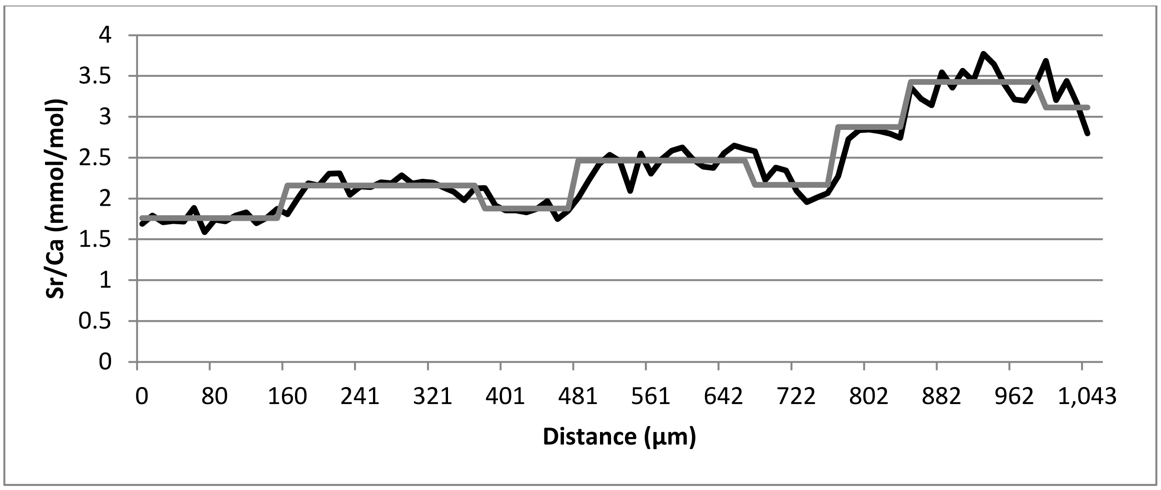


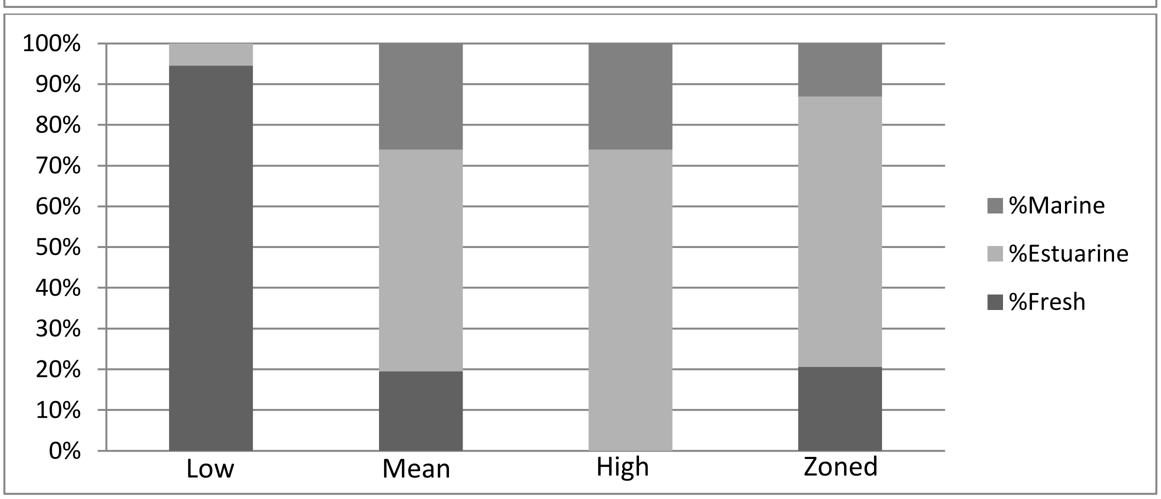
Figure AB.113. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 150. Figure 113.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.114. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 152. Figure 114.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

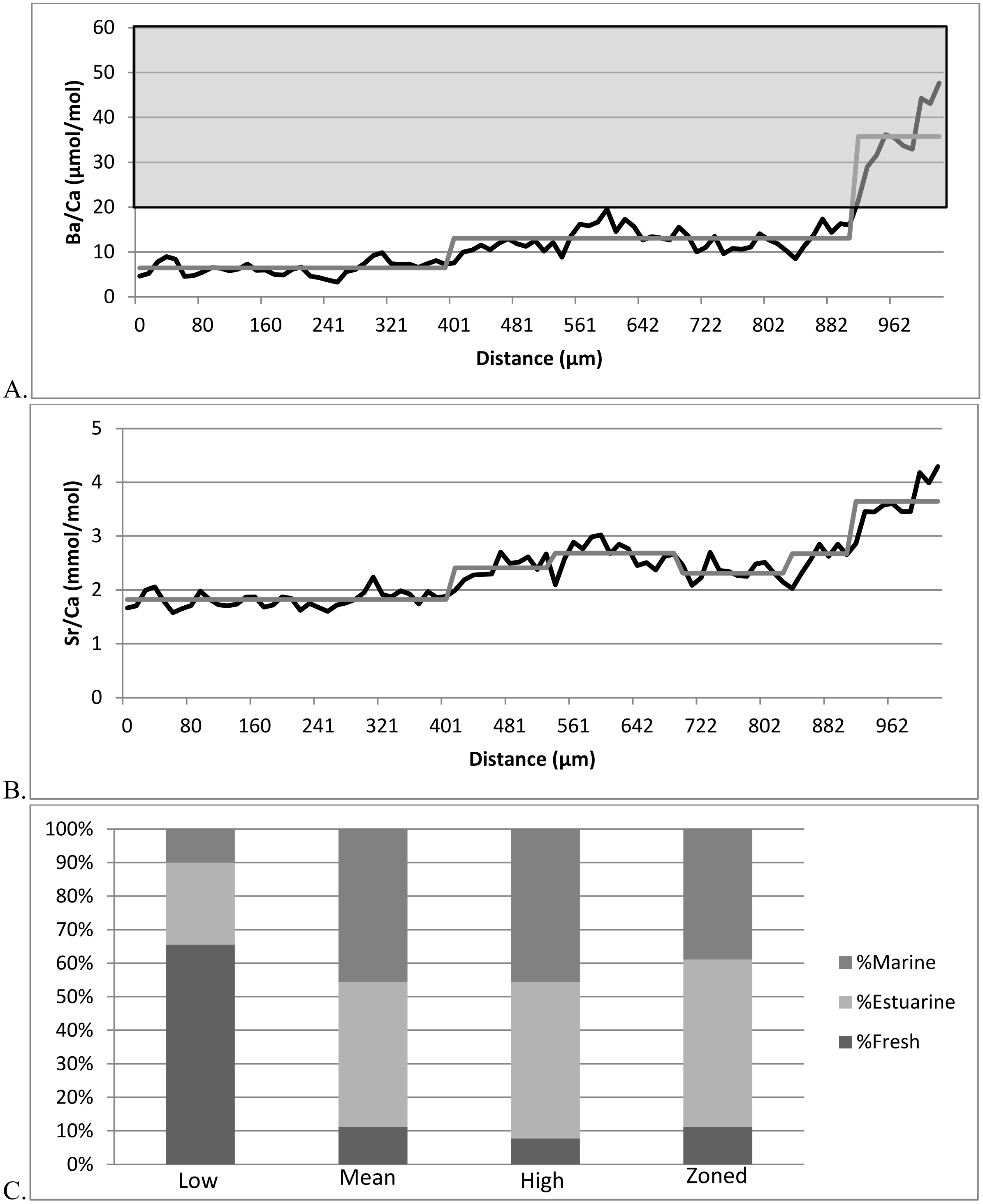
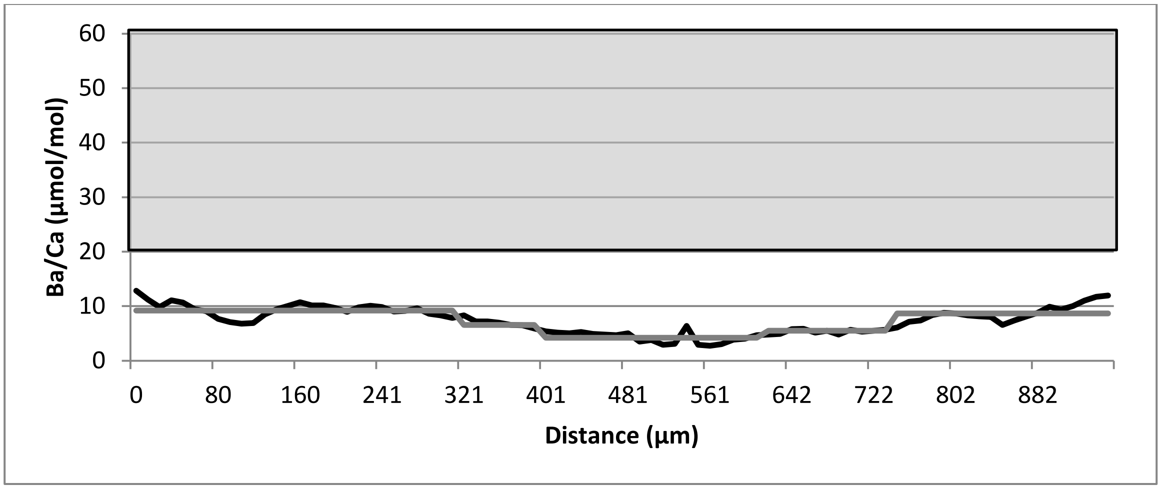
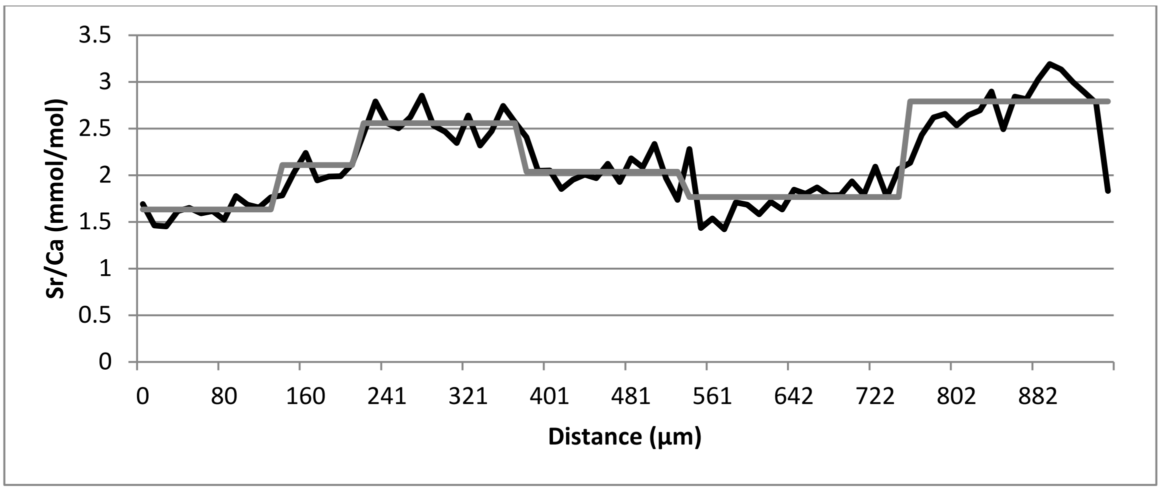


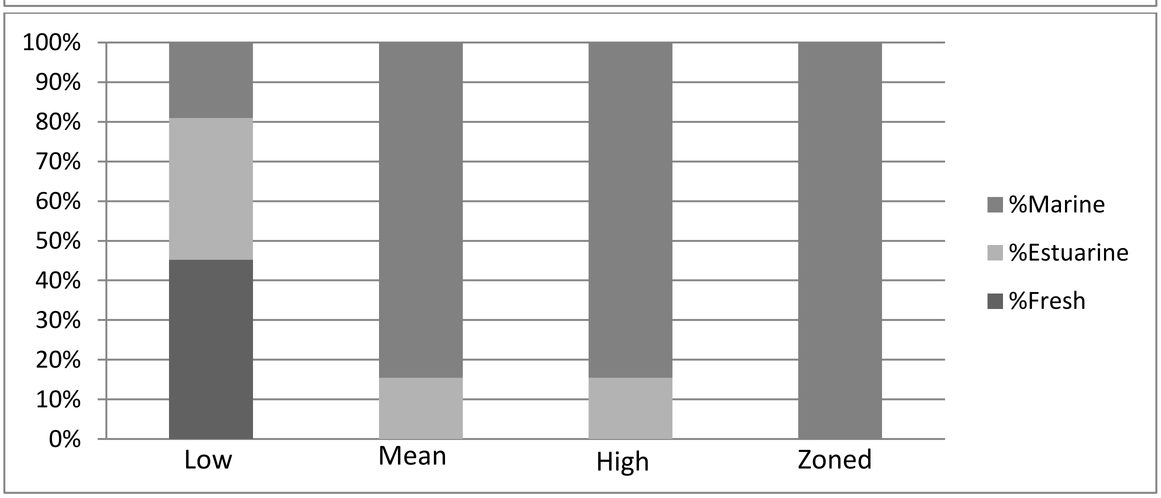
Figure AB.115. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 153. Figure 115.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

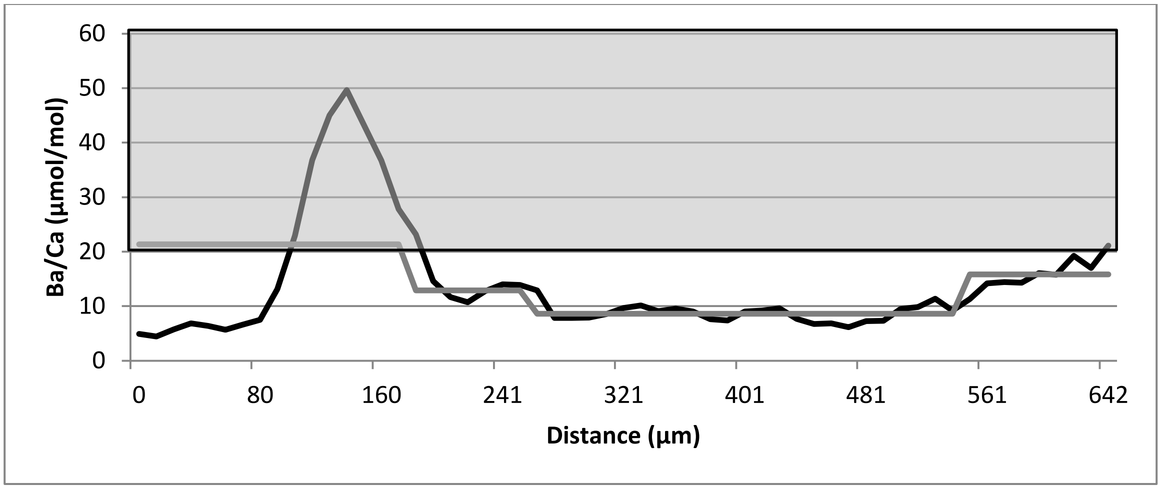


B.

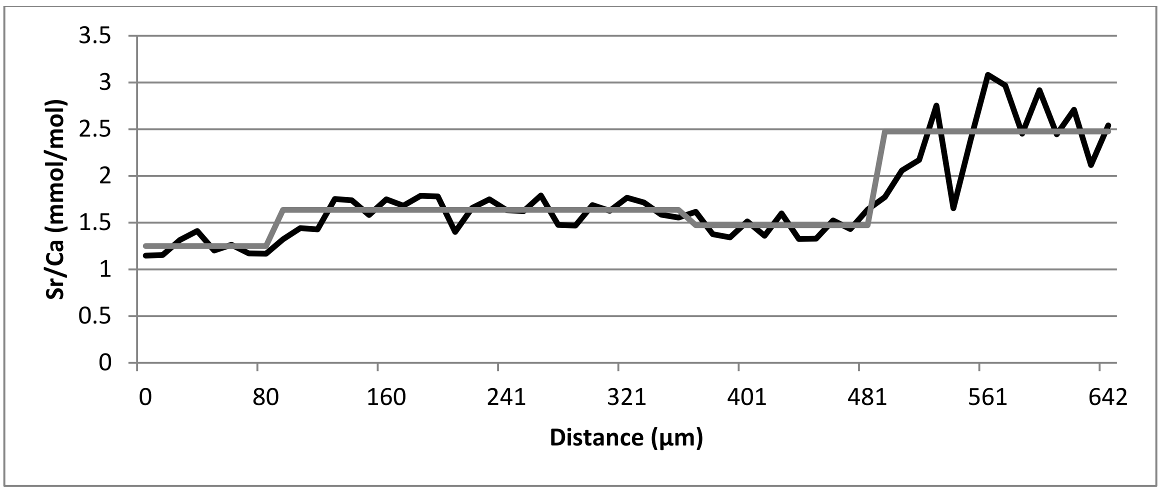


C.

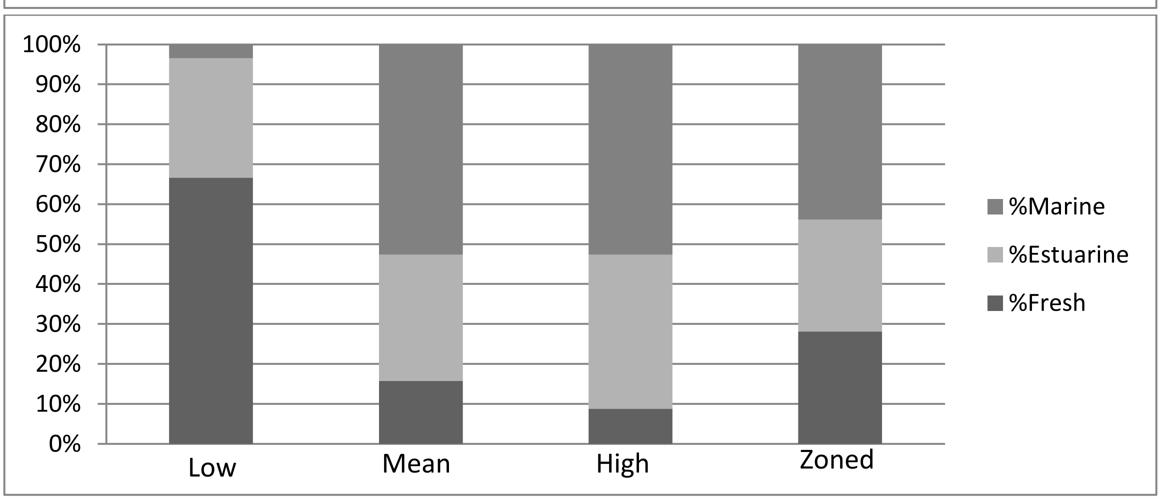
Figure AB.116. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 154. Figure 116.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

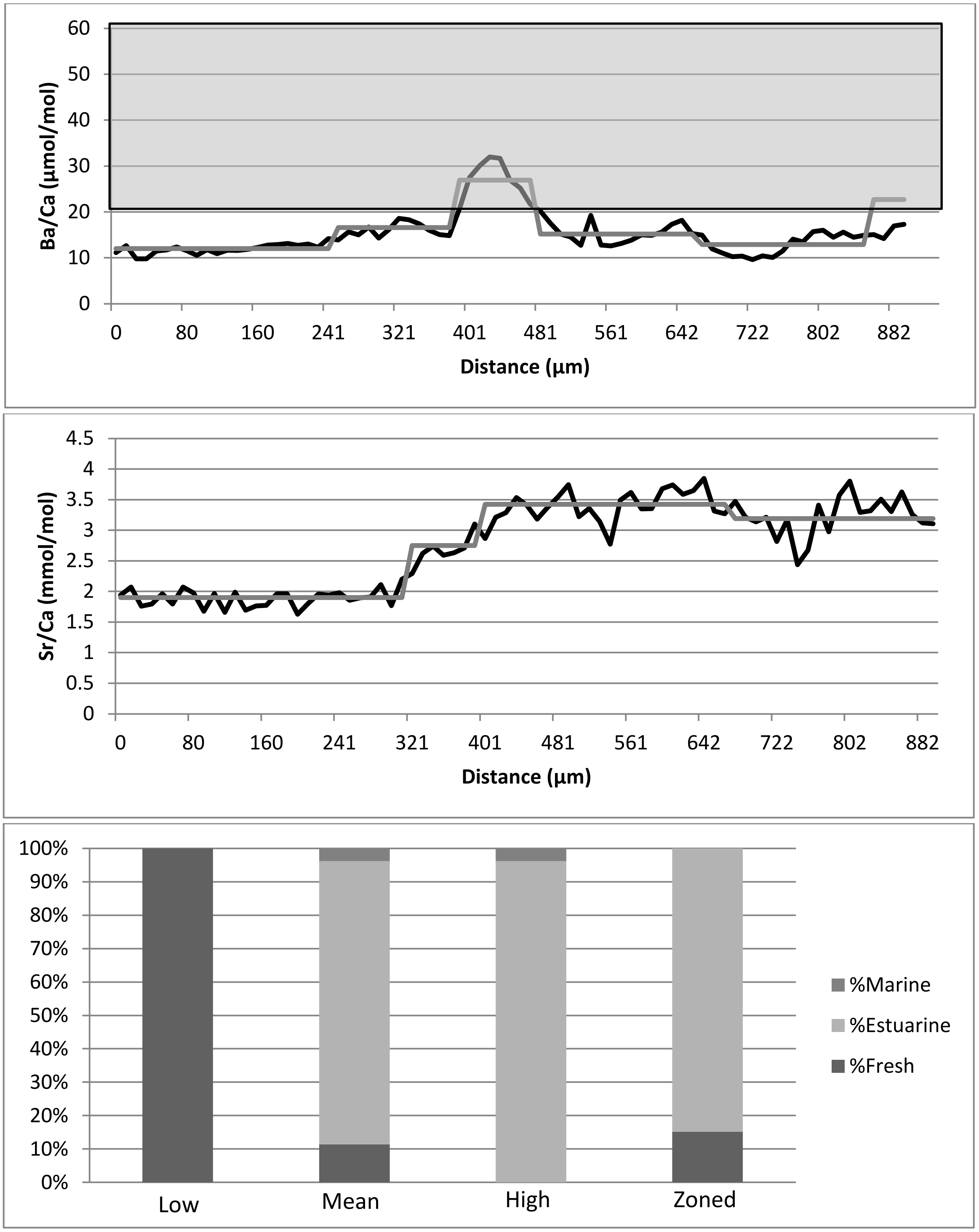


B.



C.

Figure AB.117. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 155. Figure 117.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

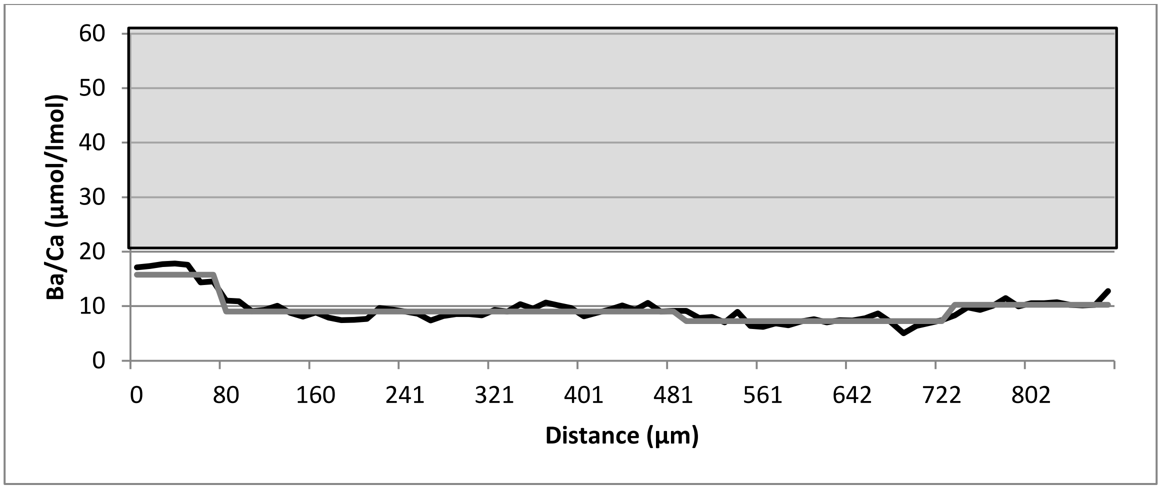


A.

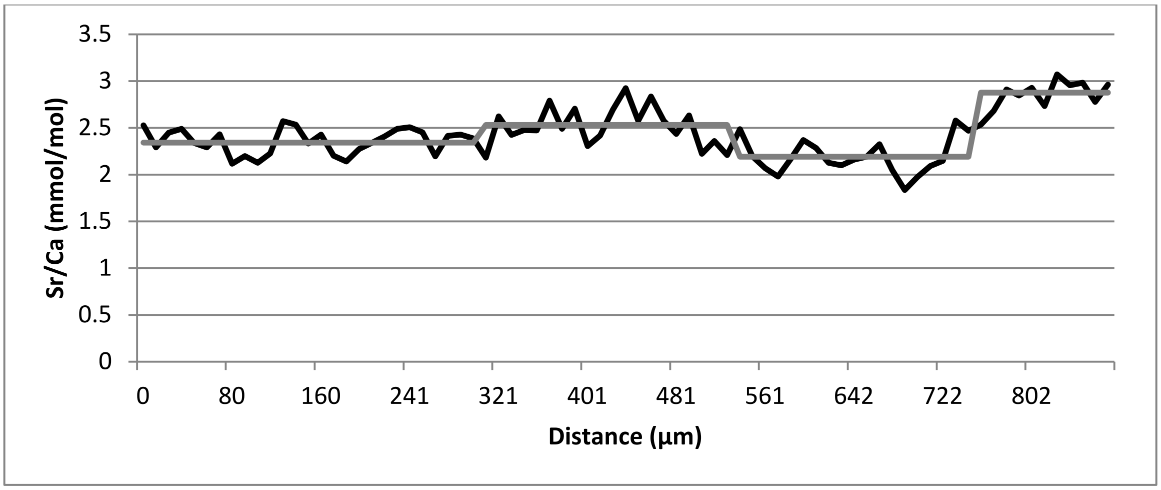
B.

C.

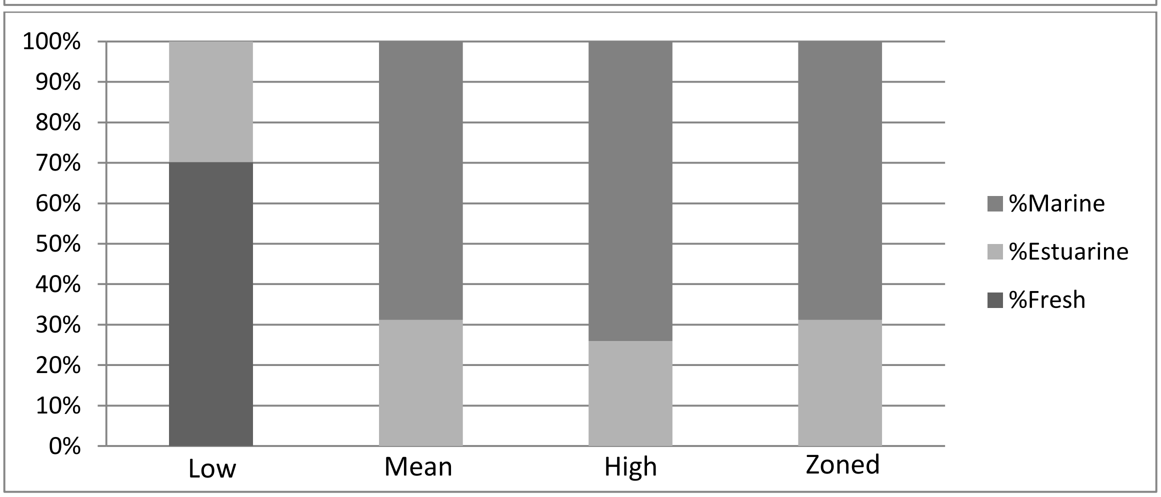
Figure AB.118. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 156. Figure 118.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

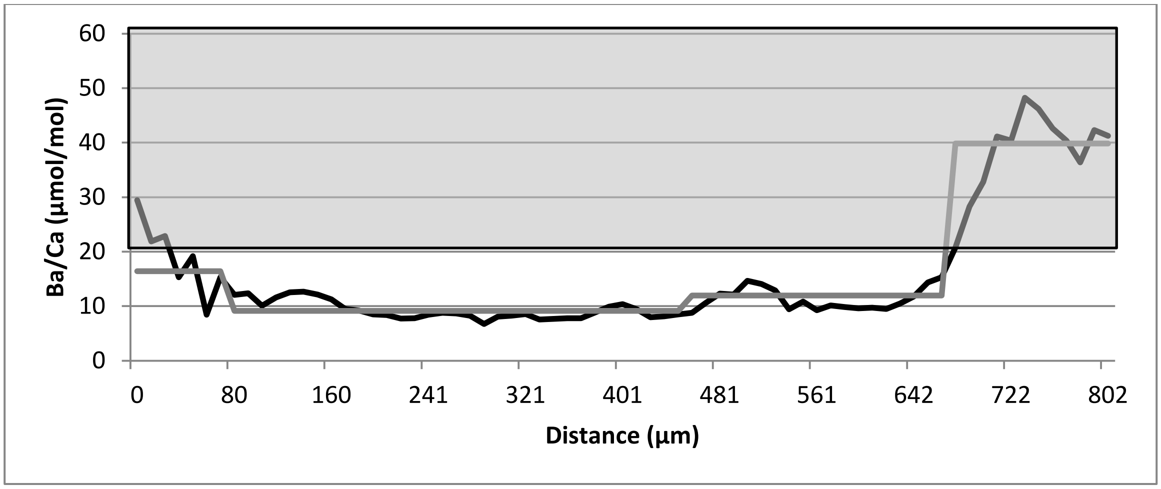


B.

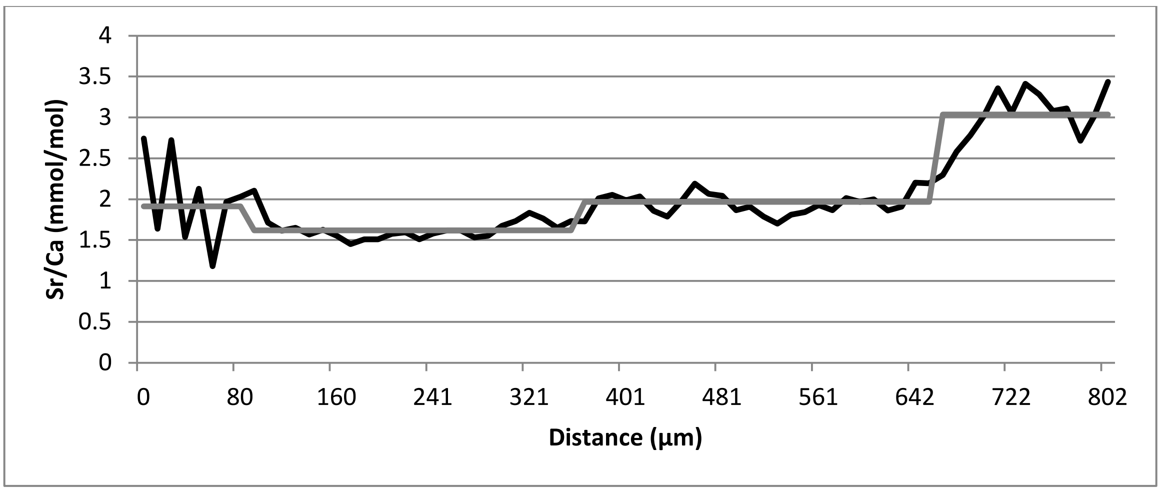


C.

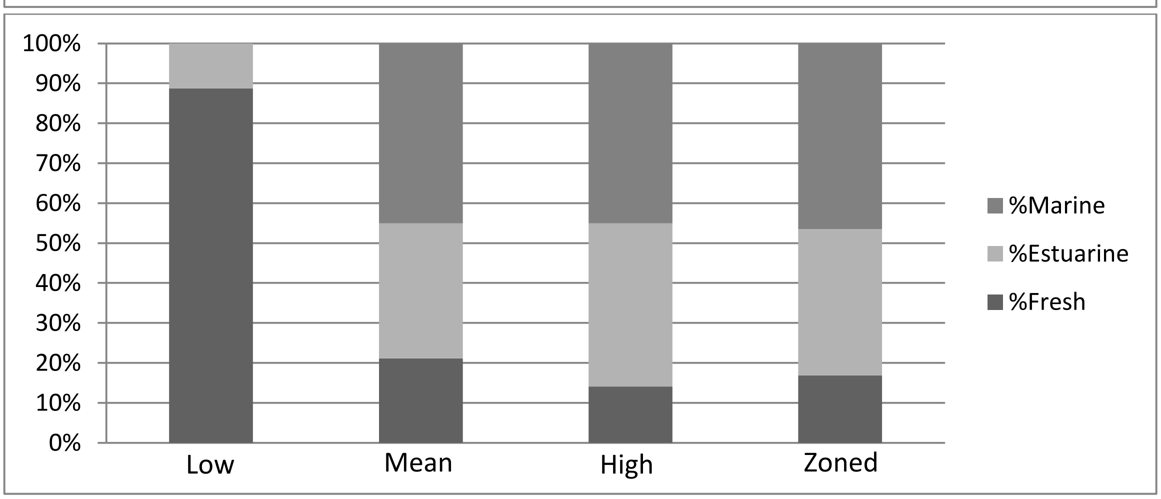
Figure AB.119. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 158. Figure 119.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

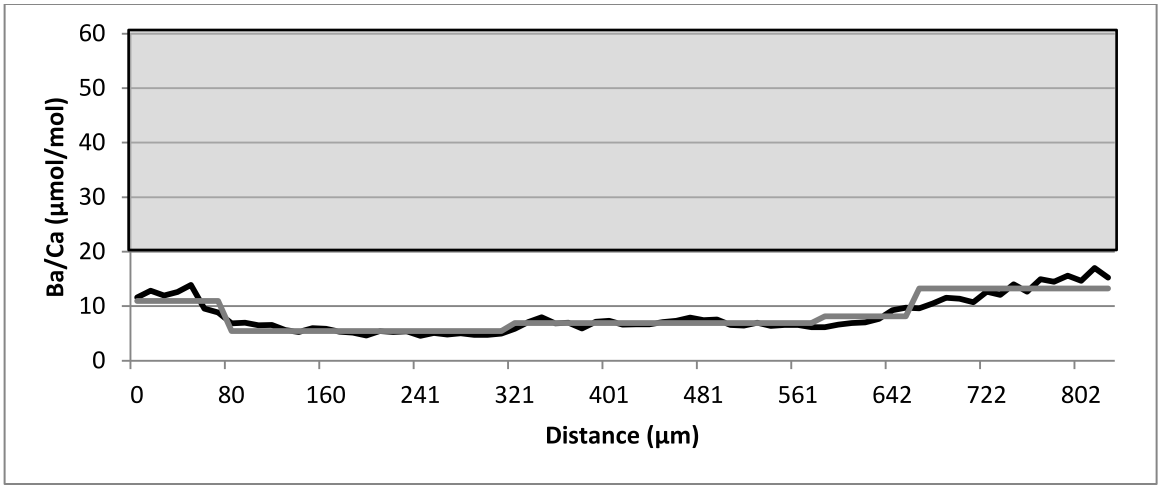


B.

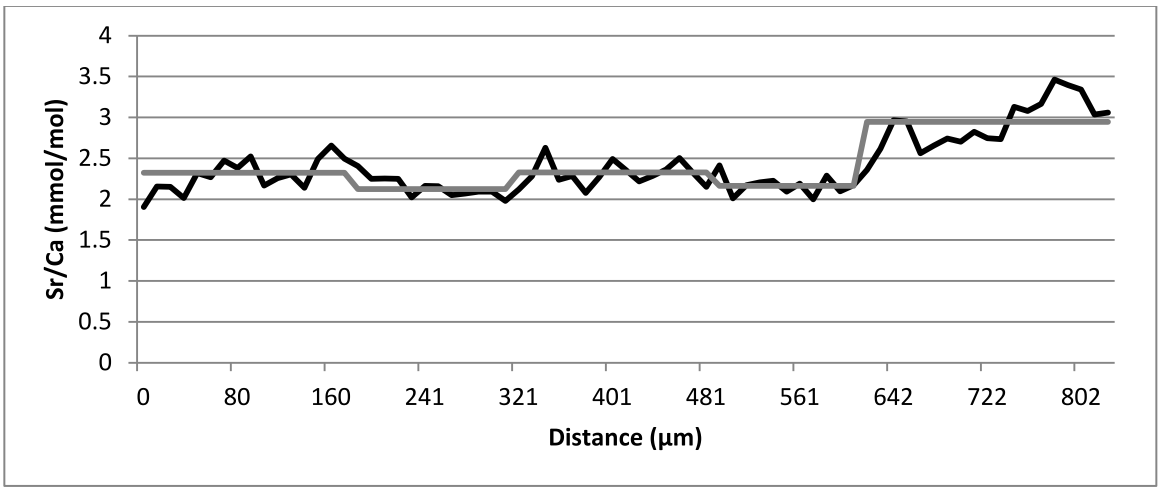


C.

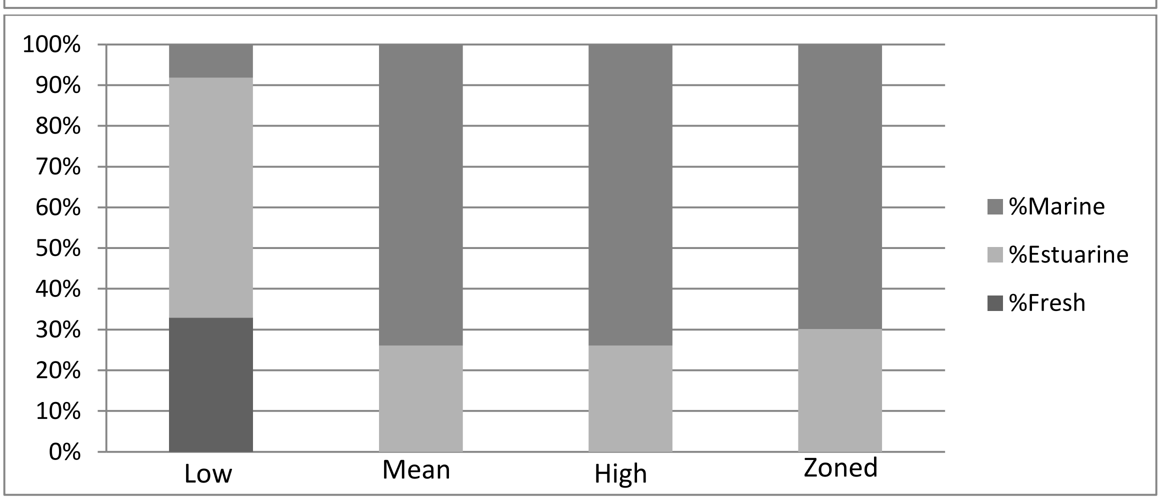
Figure AB.120. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 159. Figure 120.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



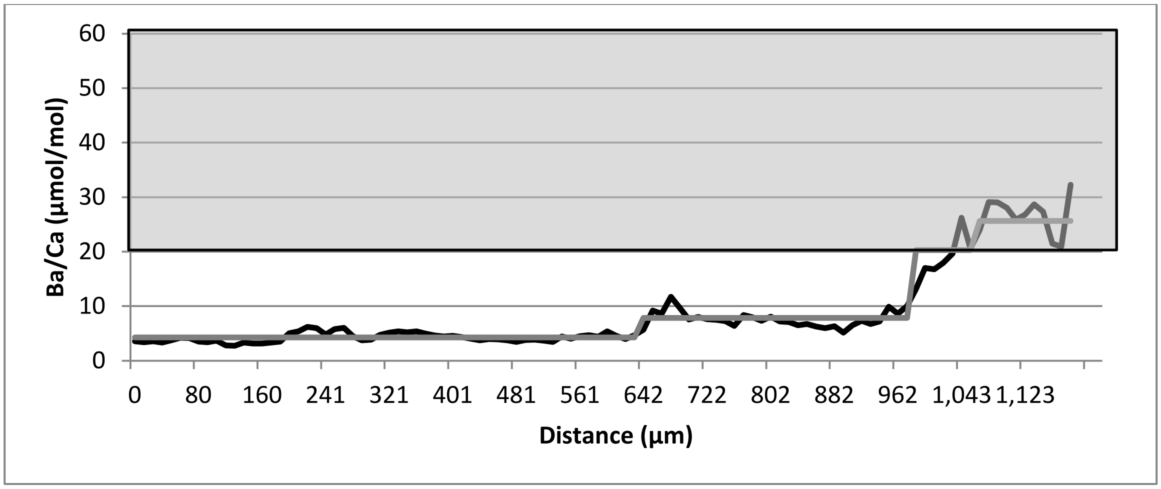
B.



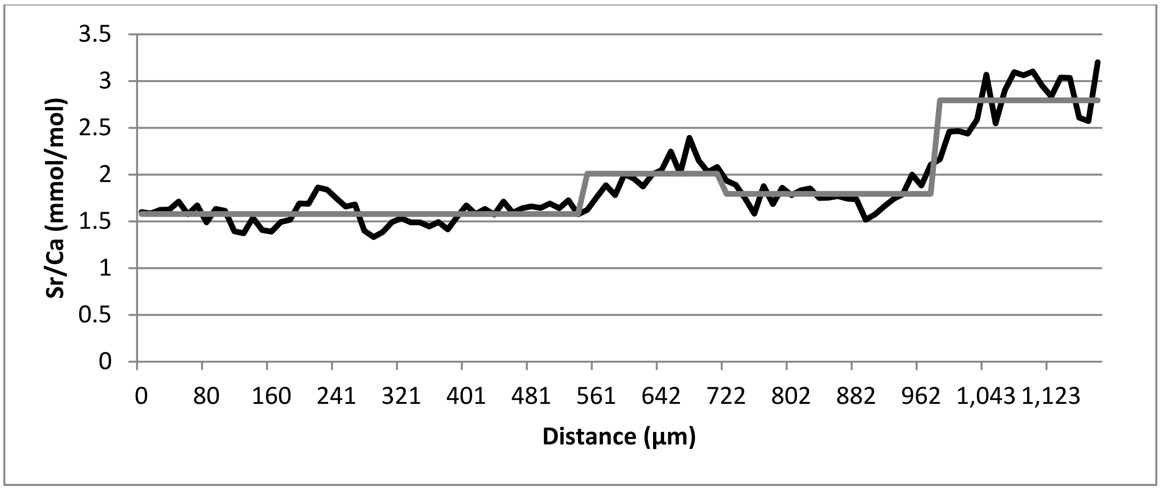
C.

Figure AB.121. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 160. Figure 121.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.



B.



C.

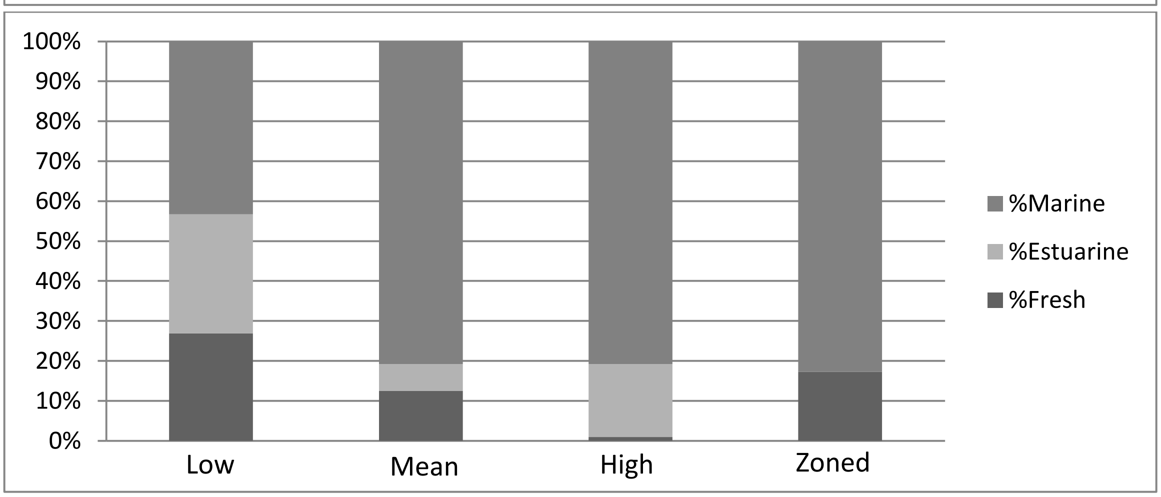


Figure AB.122. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 161. Figure 122.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

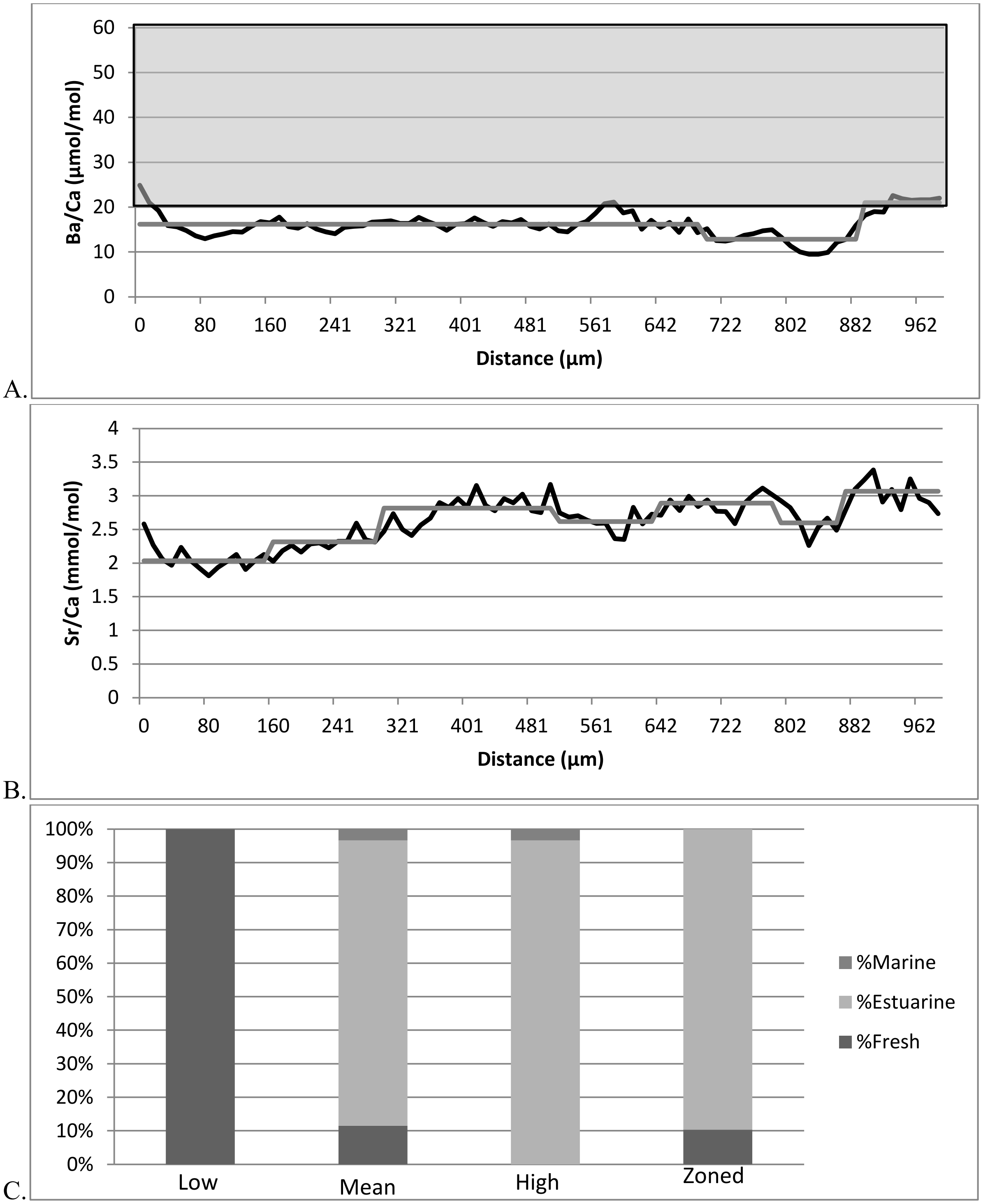
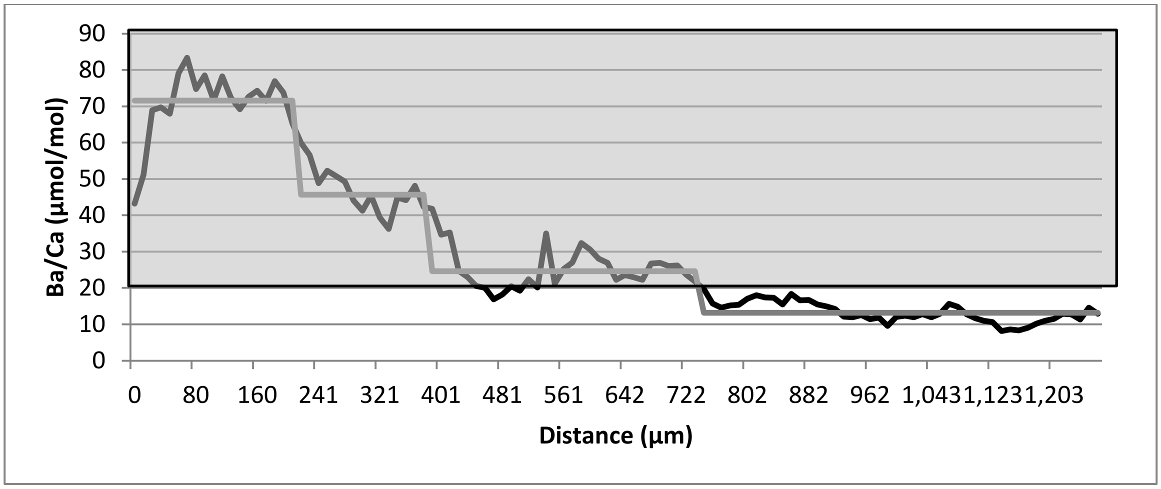
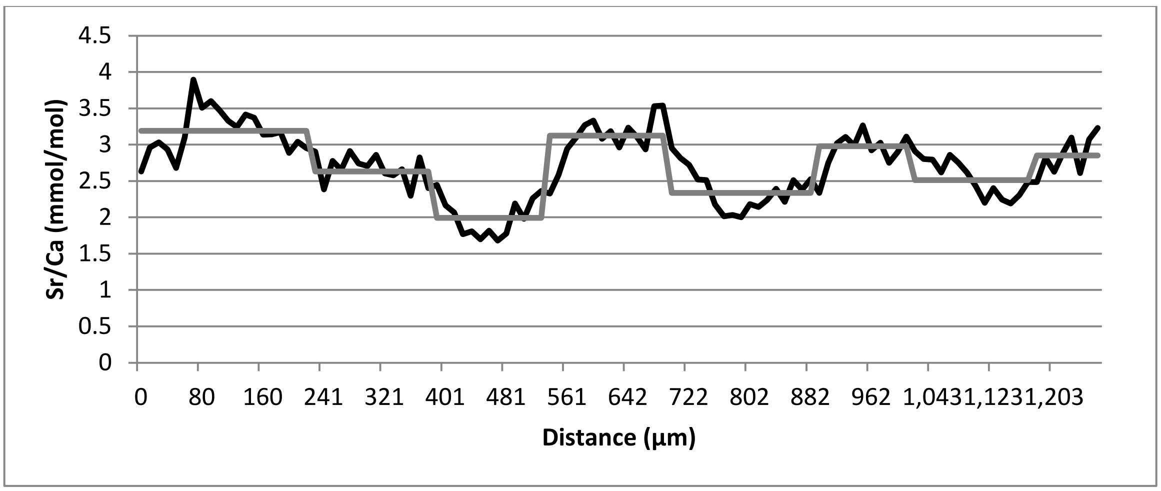


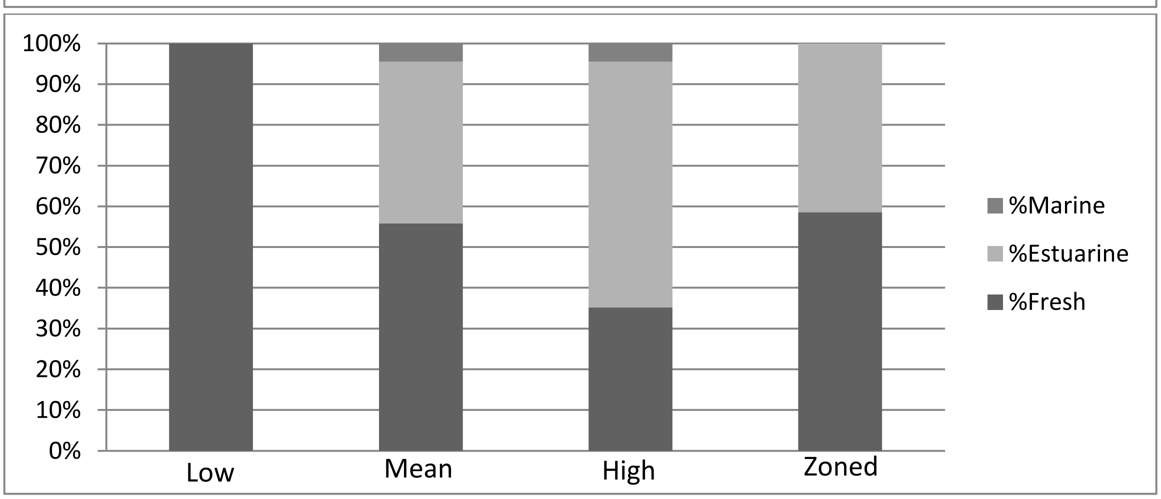
Figure AB.123. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 175. Figure 123.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

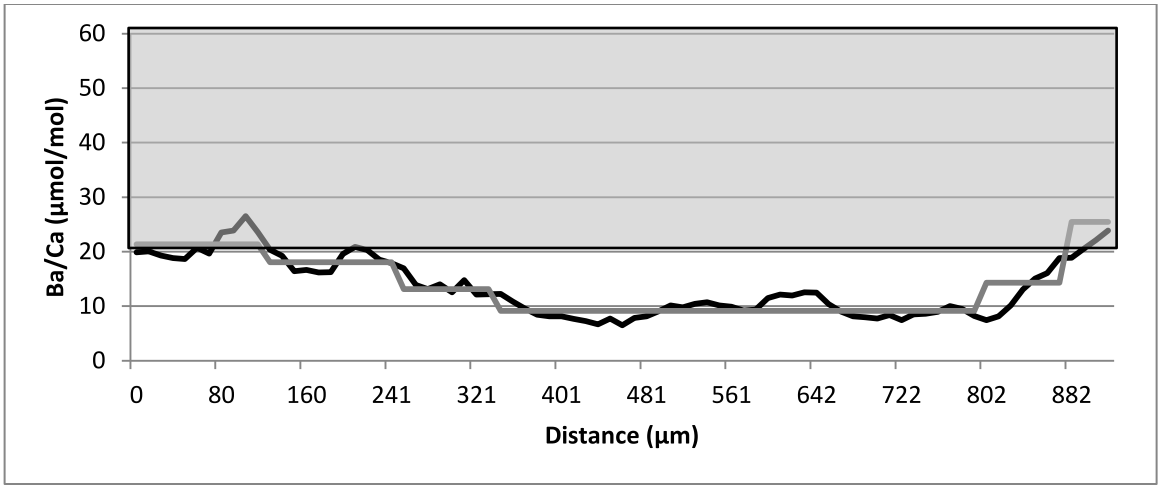


B.

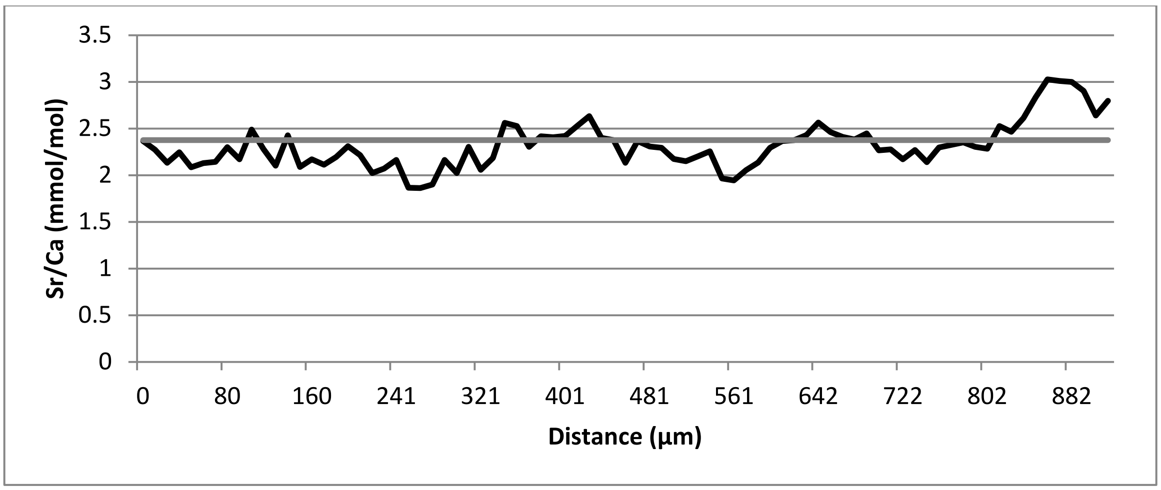


C.

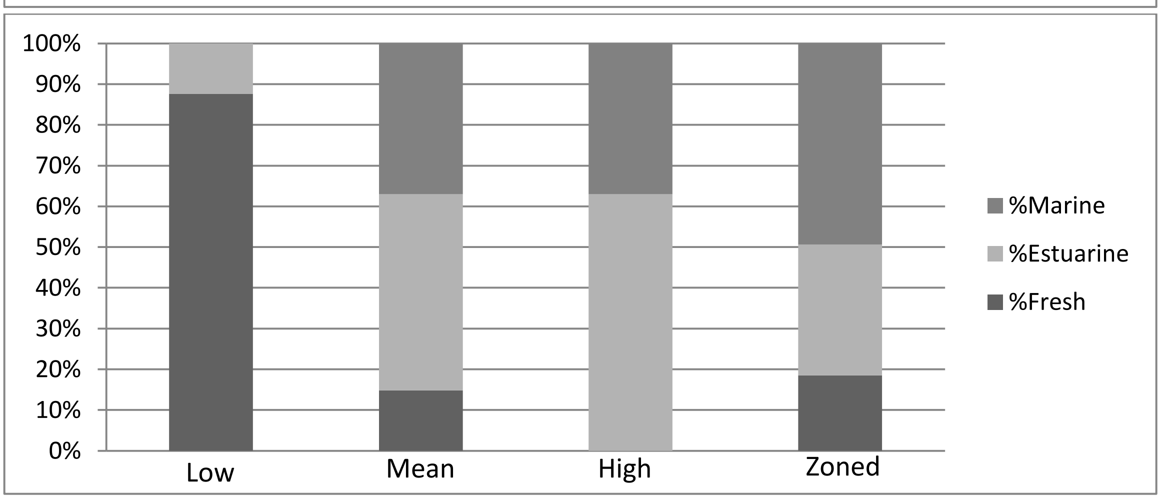
Figure AB.124. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 176. Figure 124.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

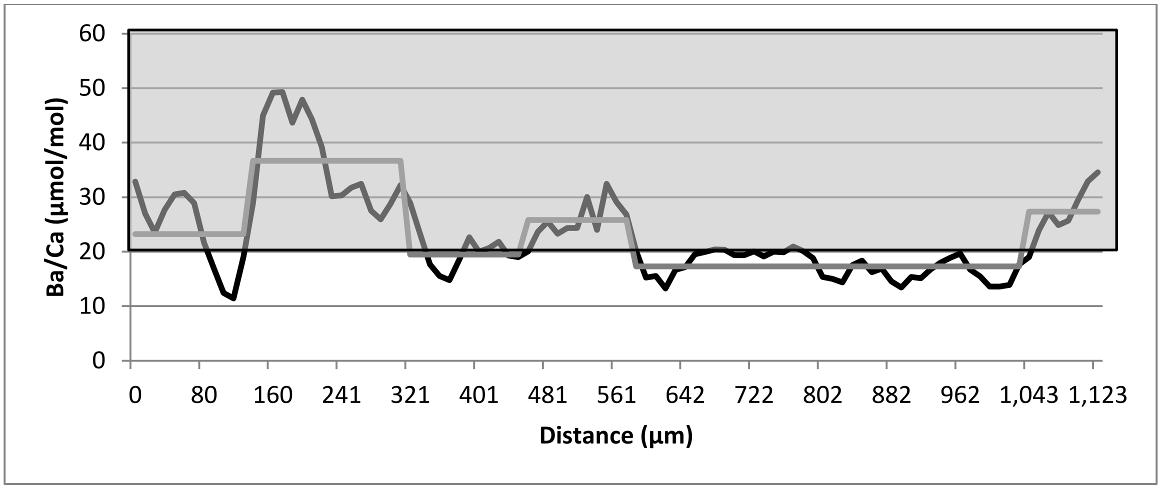


B.

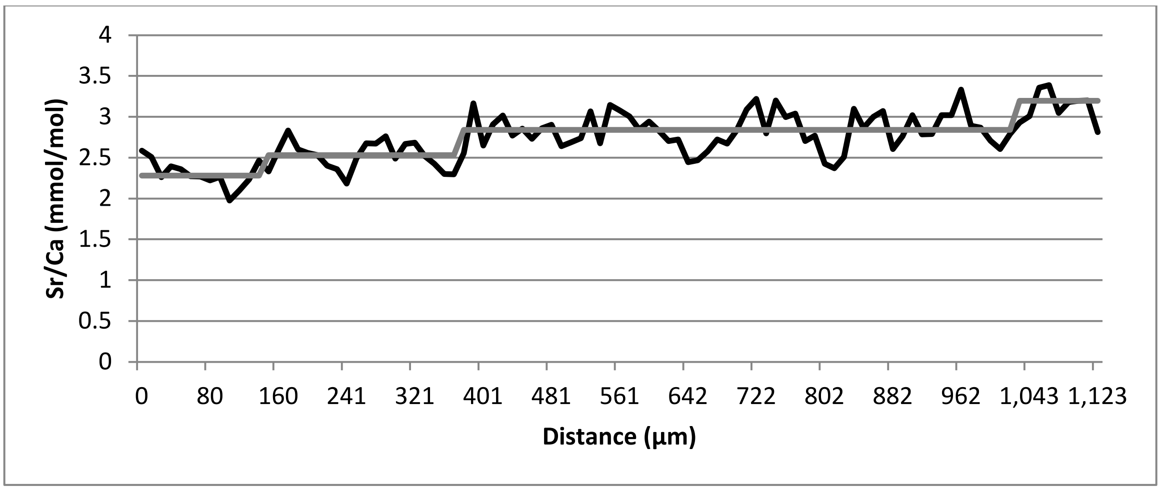


C.

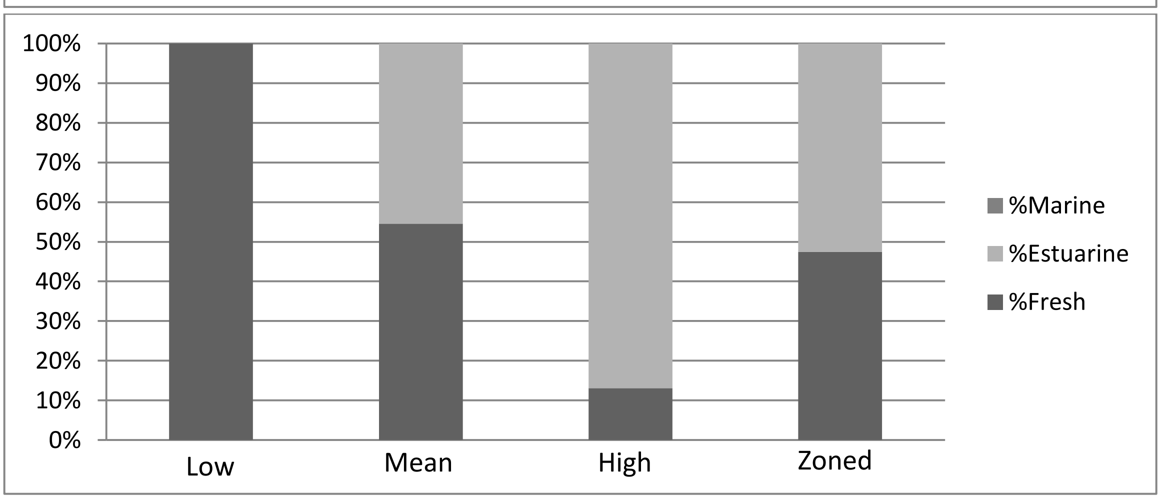
Figure AB.125. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 178. Figure 125.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.126. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 180. Figure 126.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

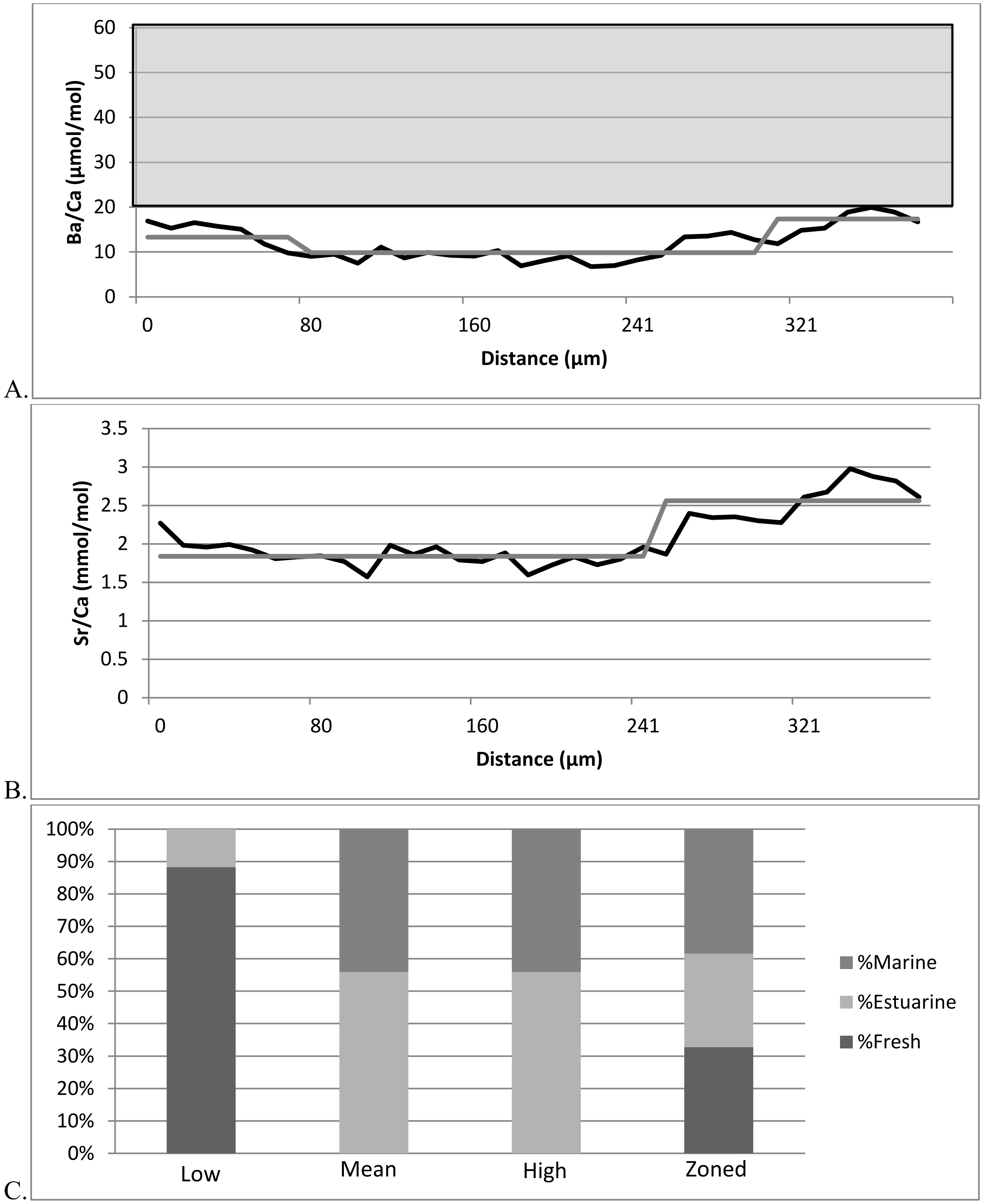
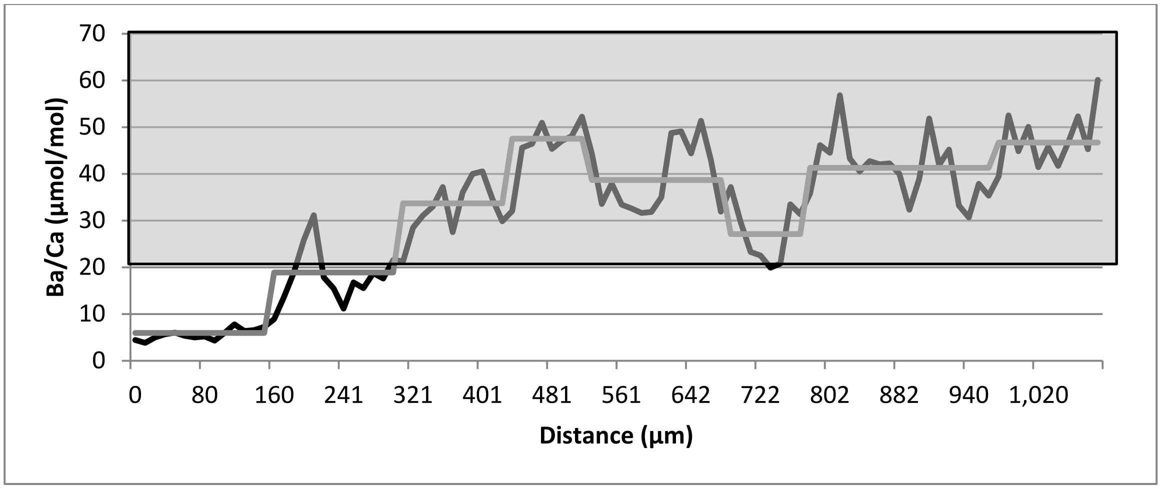
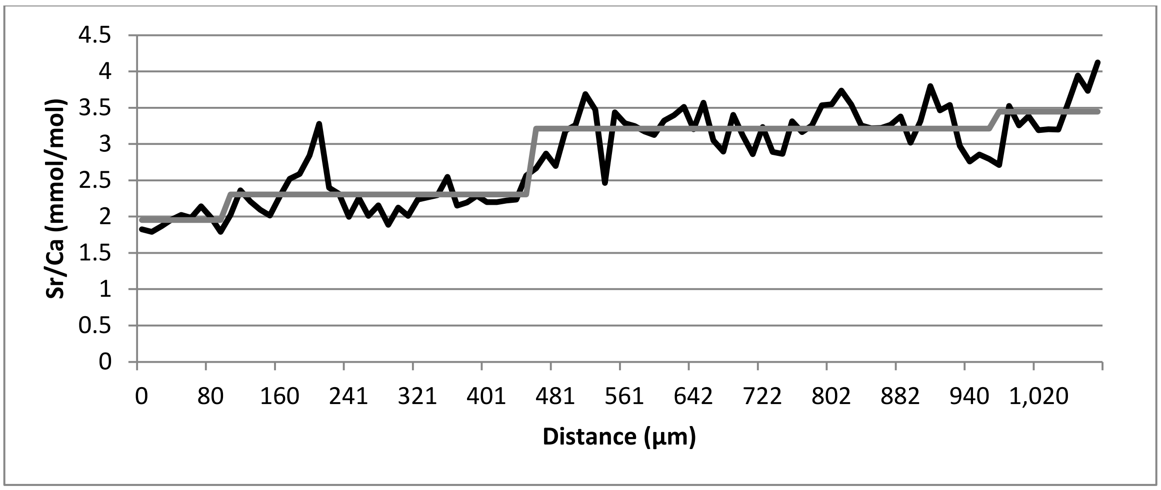


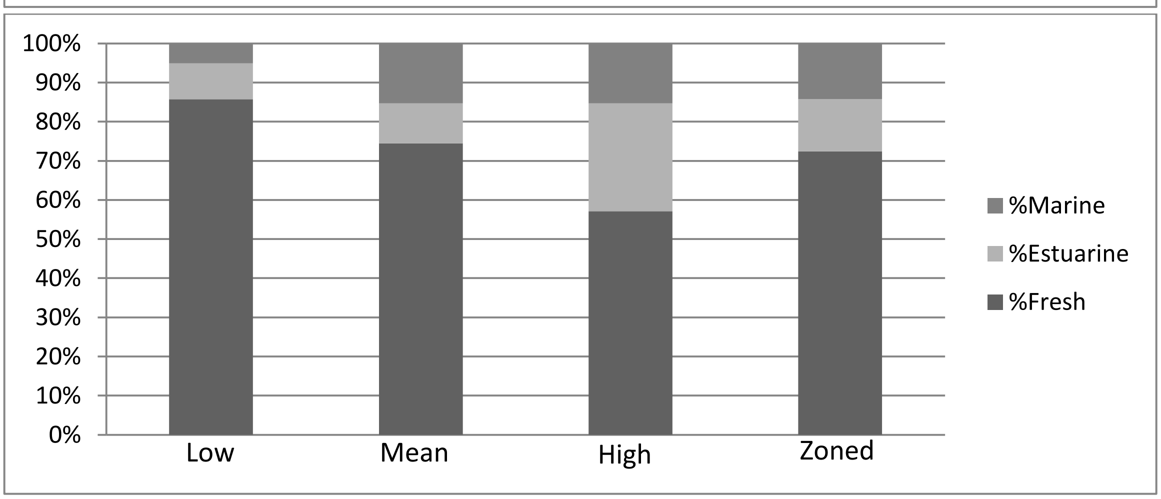
Figure AB.127. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 182. Figure 127.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.128. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 183. Figure 128.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

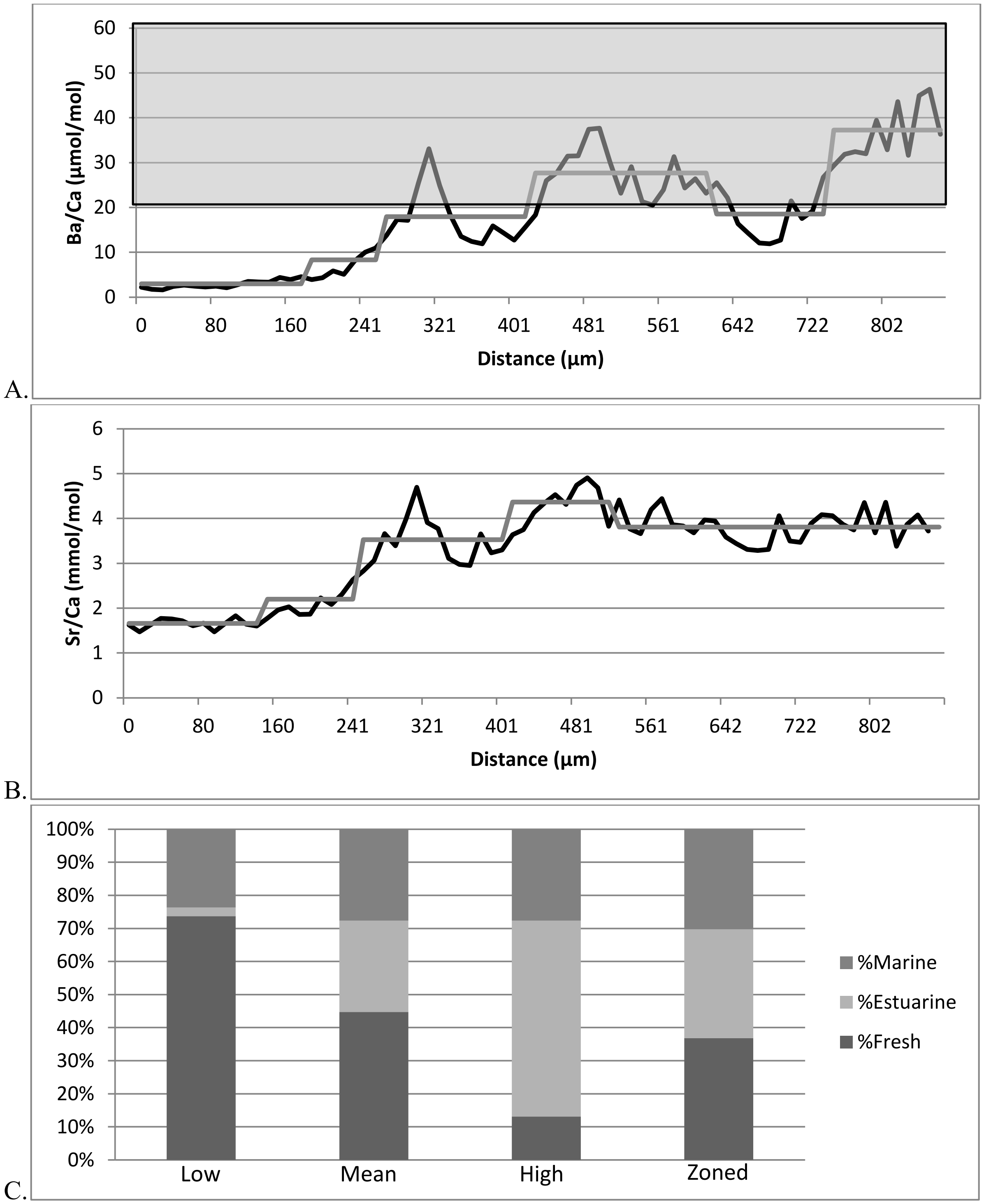


Figure AB.129. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 188. Figure 129.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

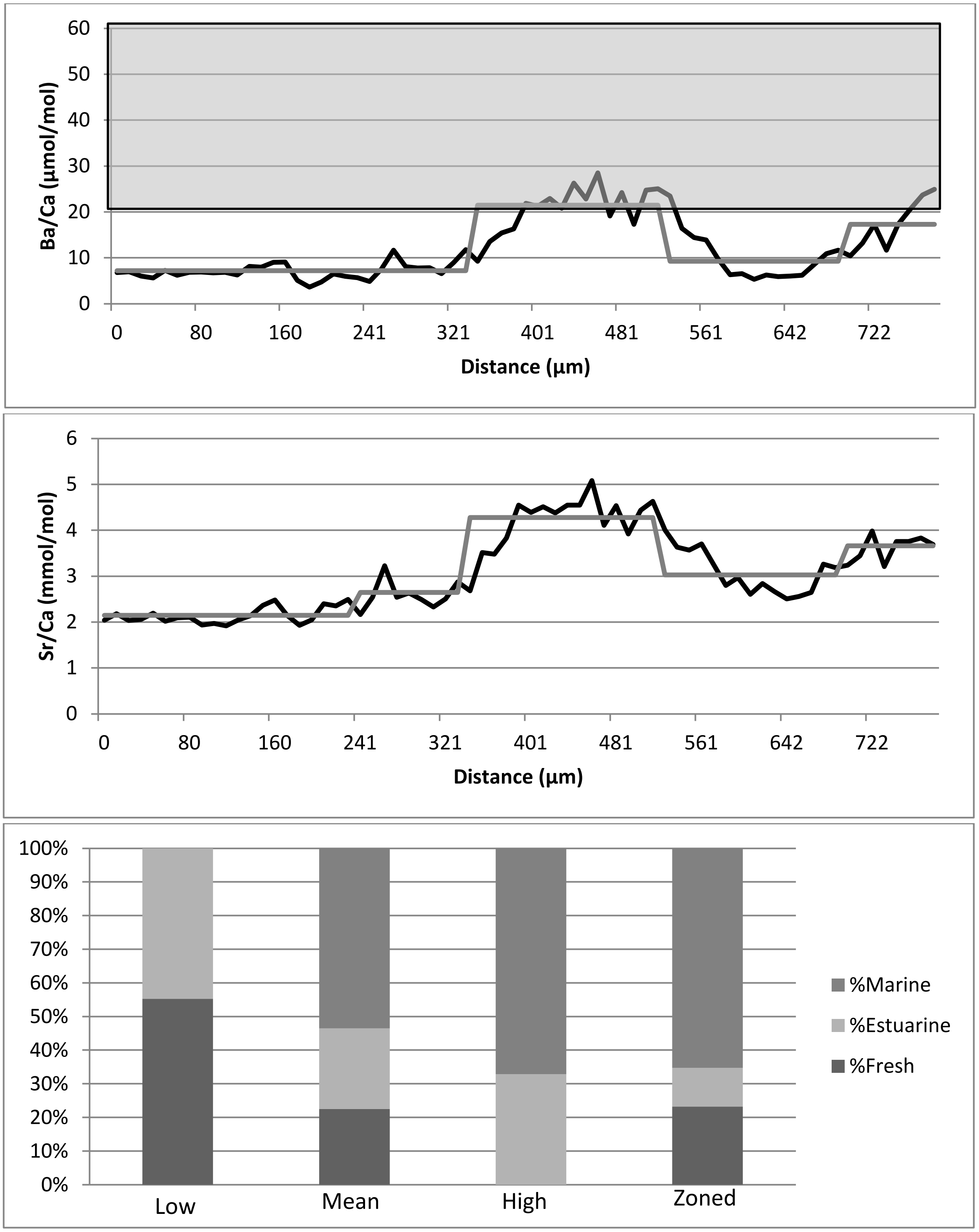
A.
B.
C.


Figure AB.130. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 189. Figure 130.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

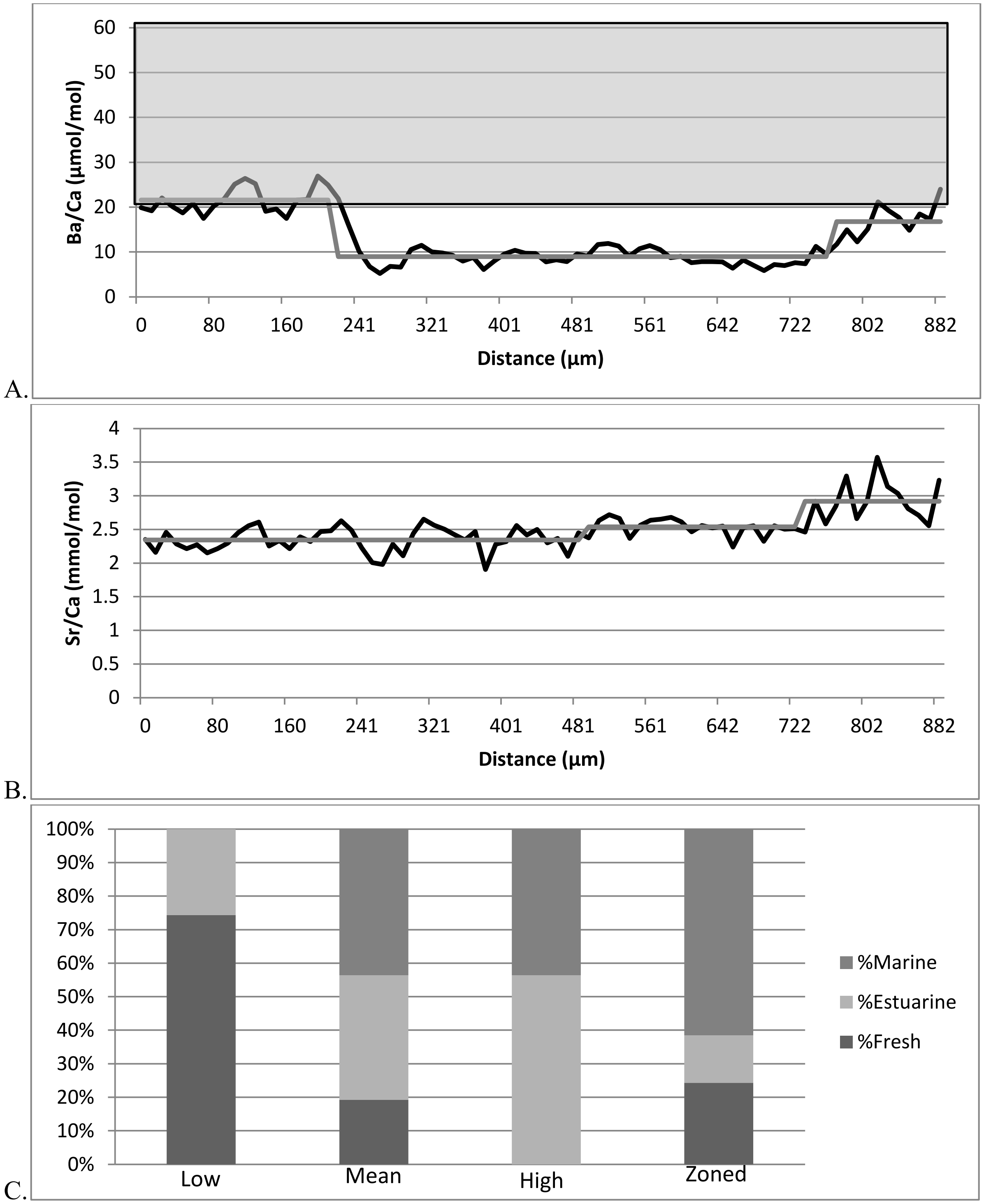
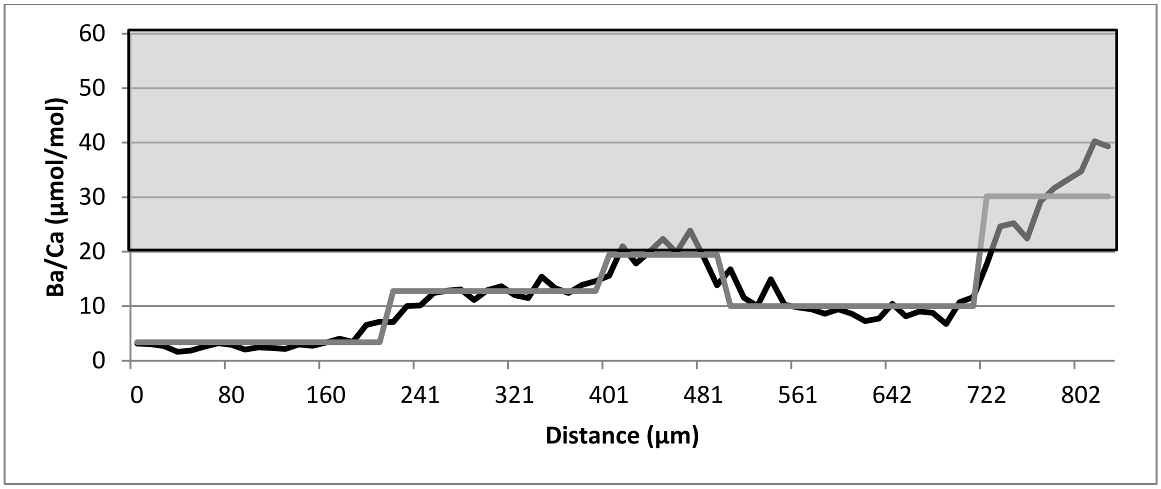
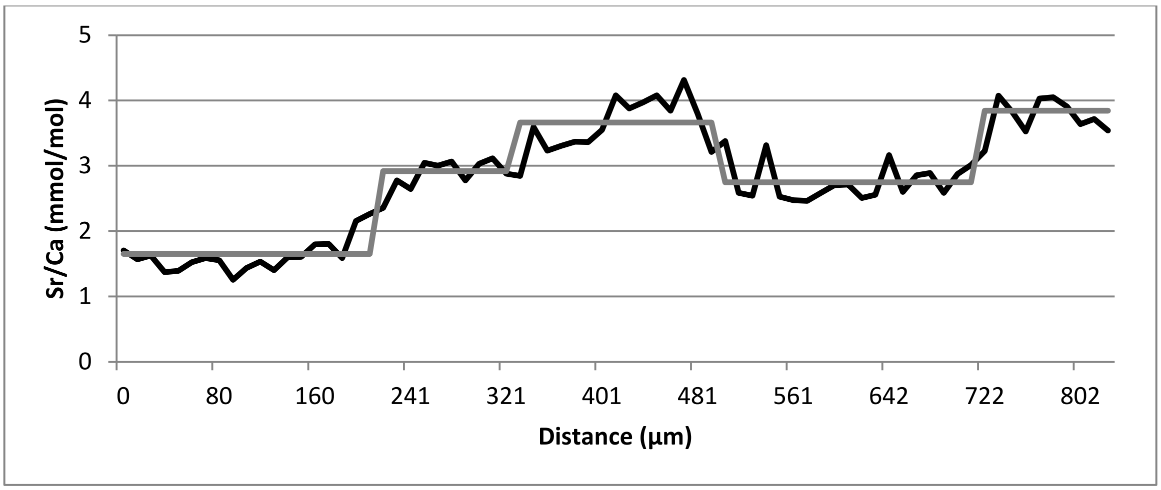


Figure AB.131. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 190. Figure 131.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


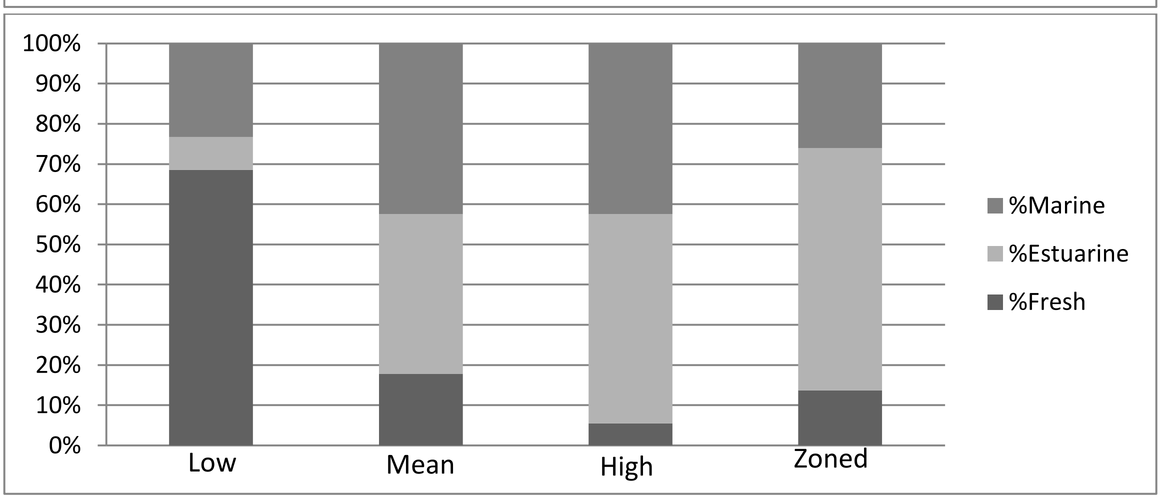
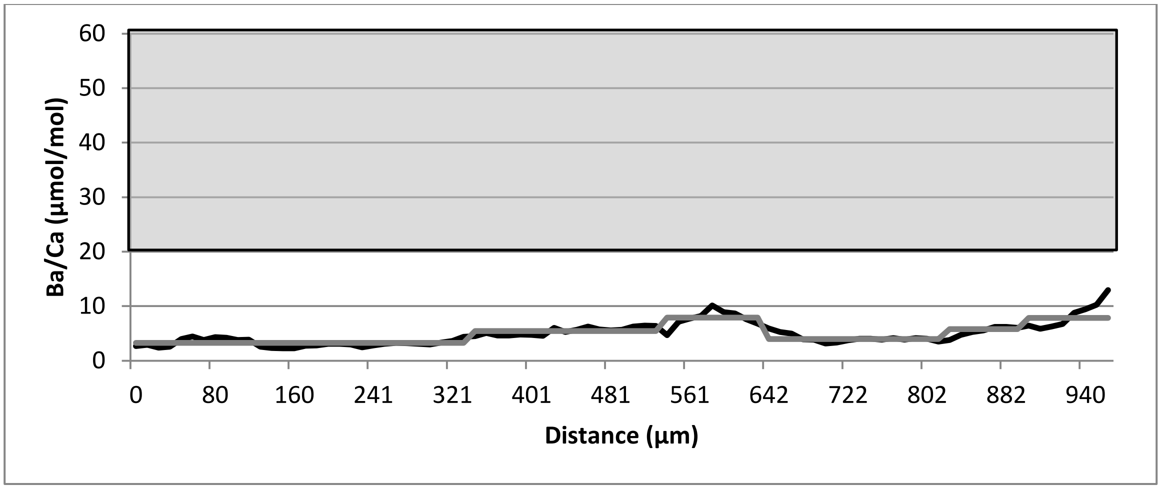
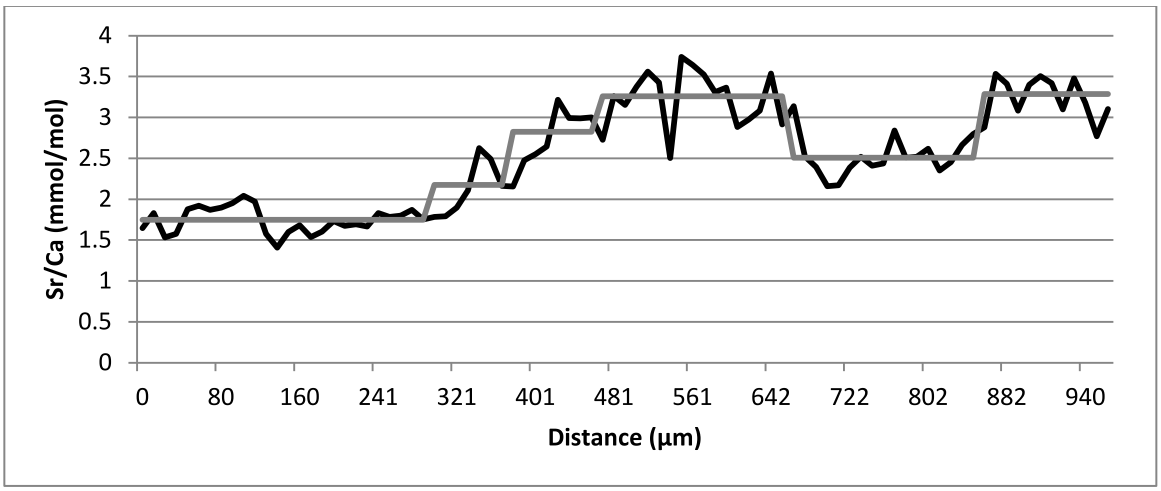
C.


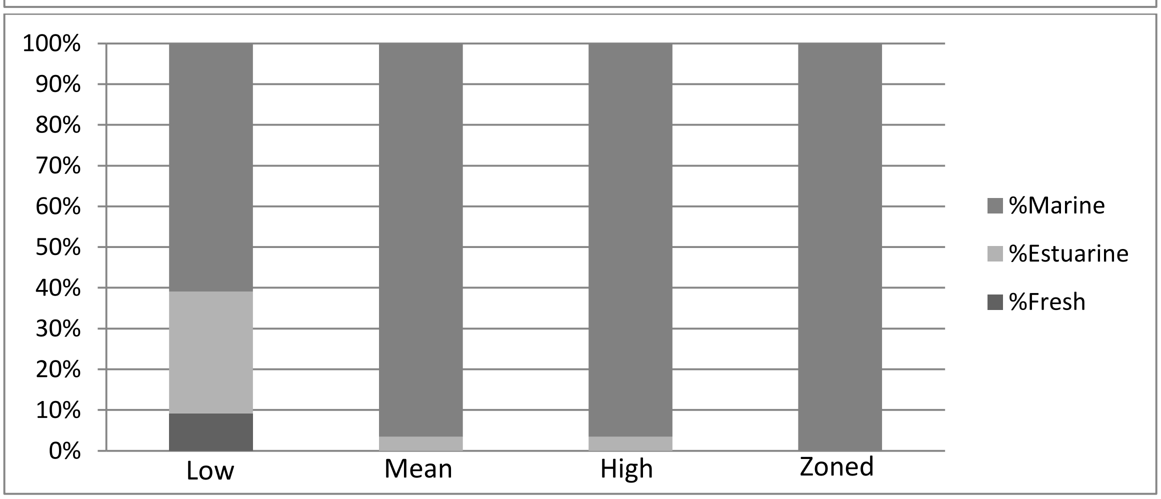
Figure AB.132. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 192. Figure 132.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.133. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 193. Figure 133.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

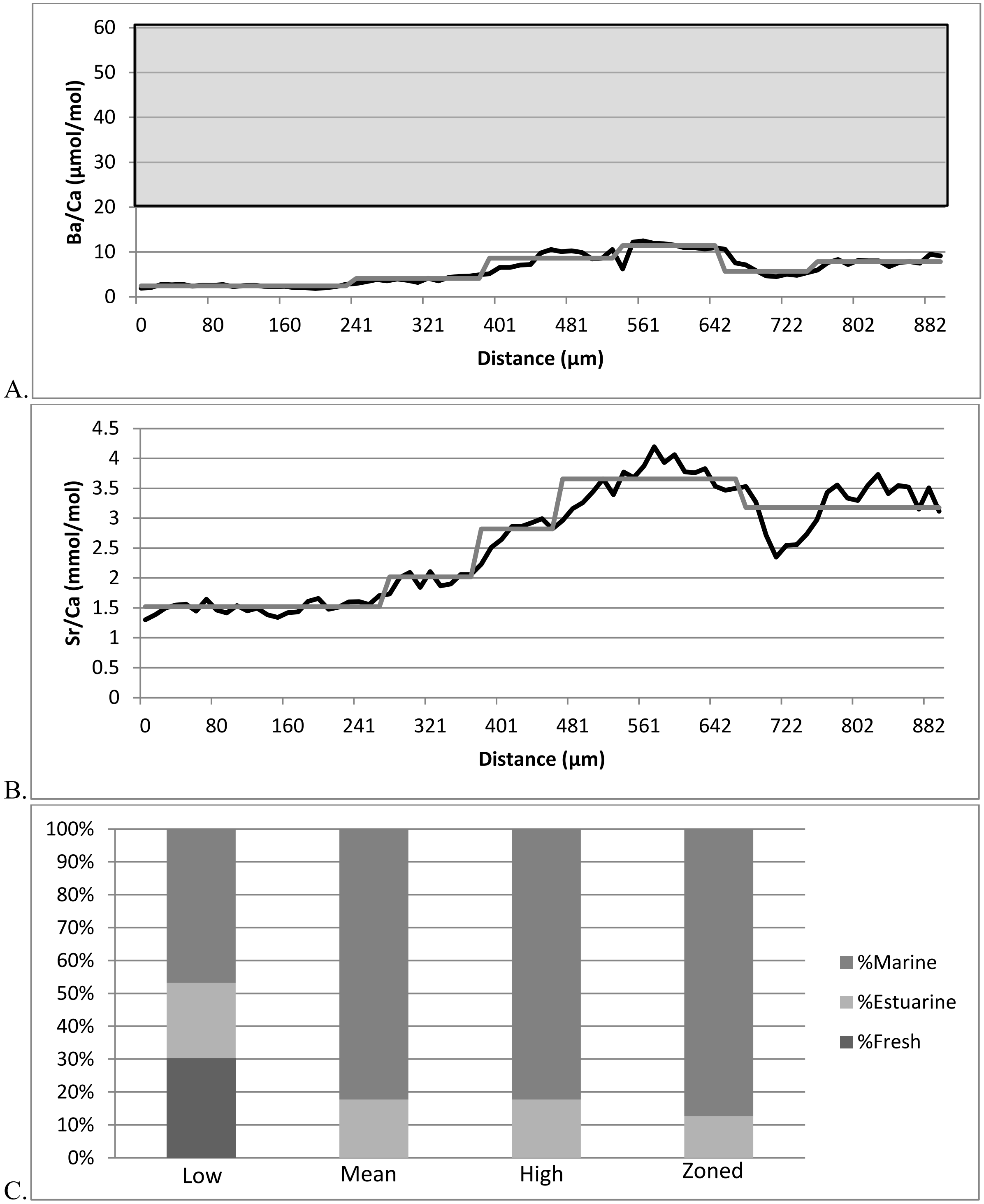


Figure AB.134. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 196. Figure 134.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

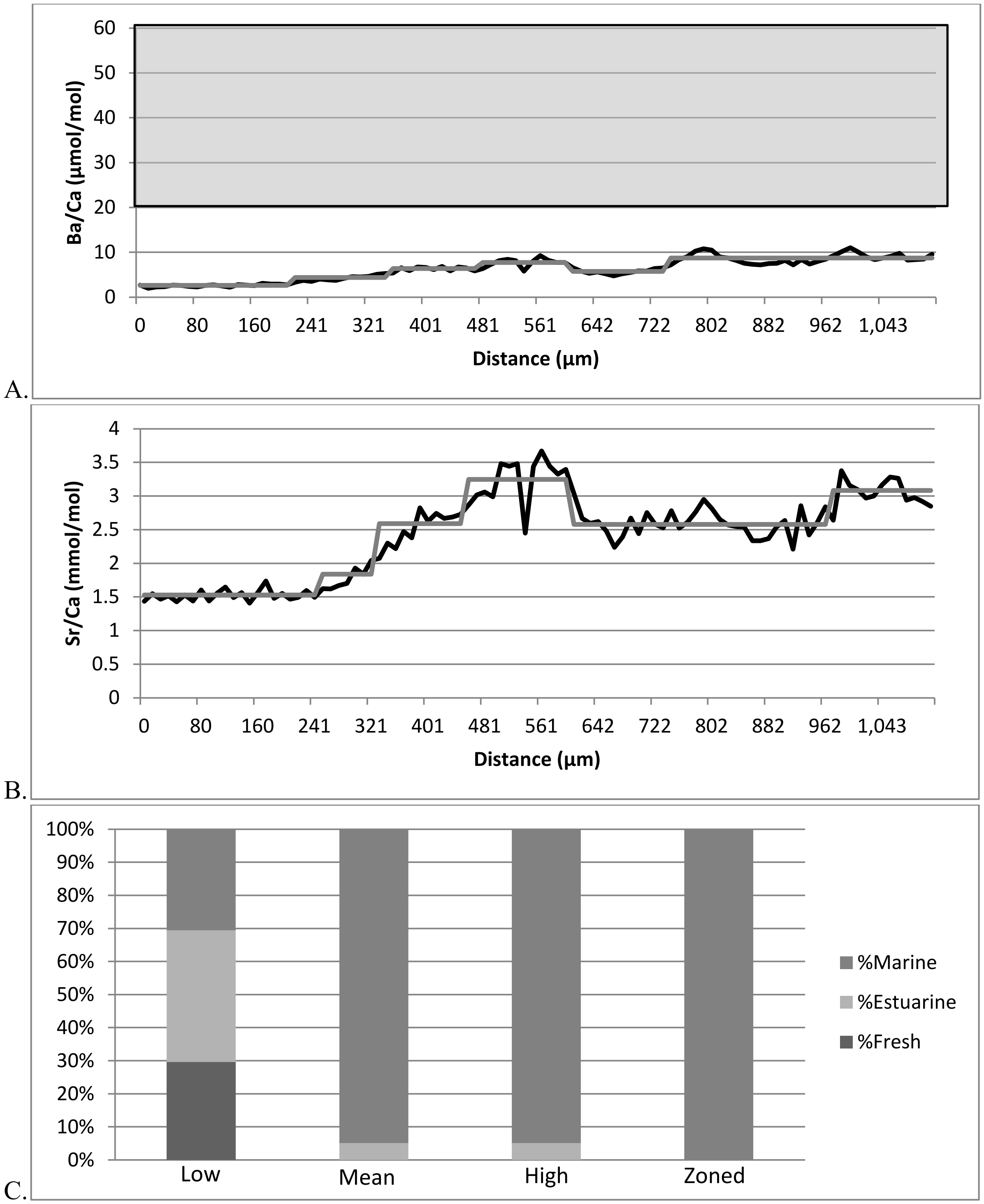
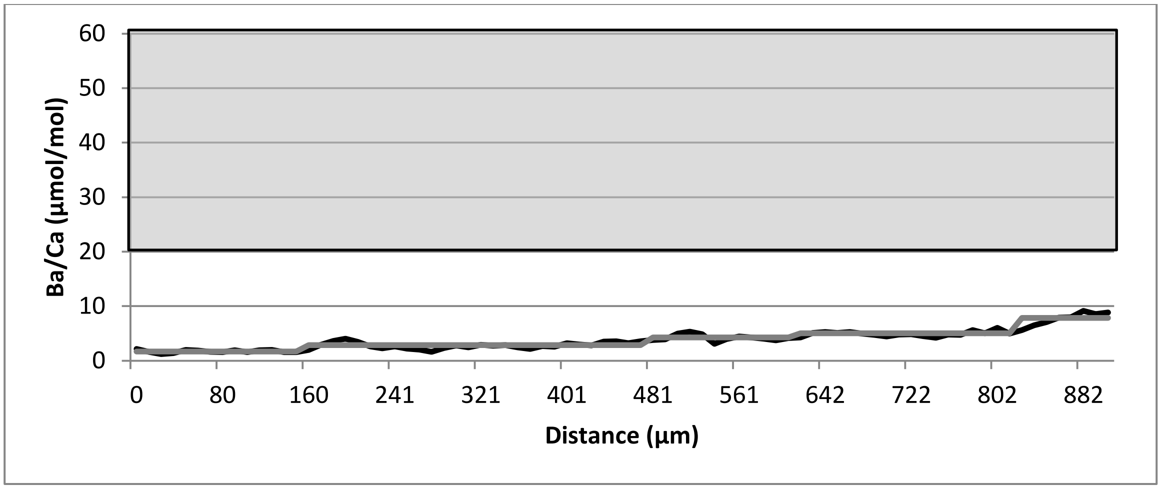
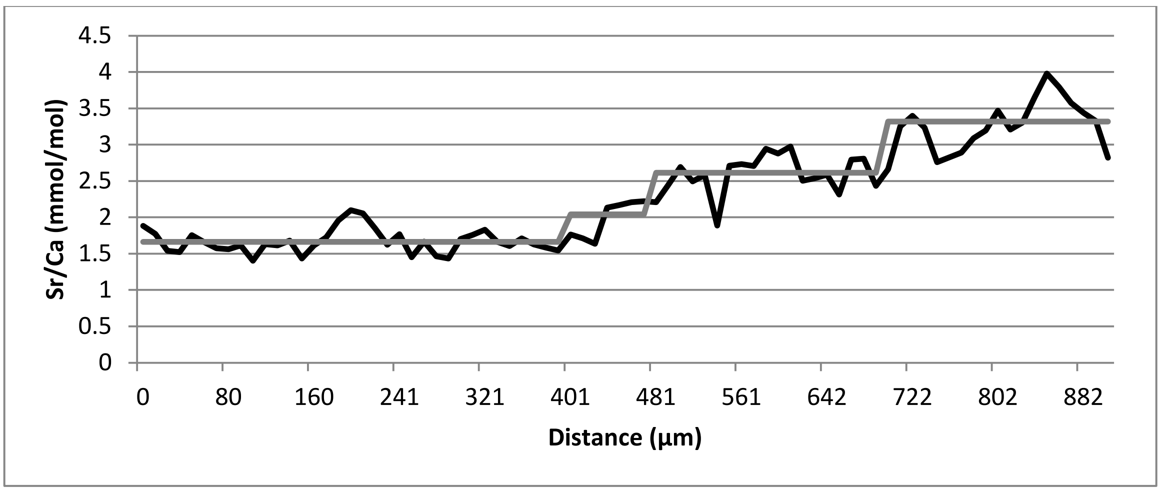


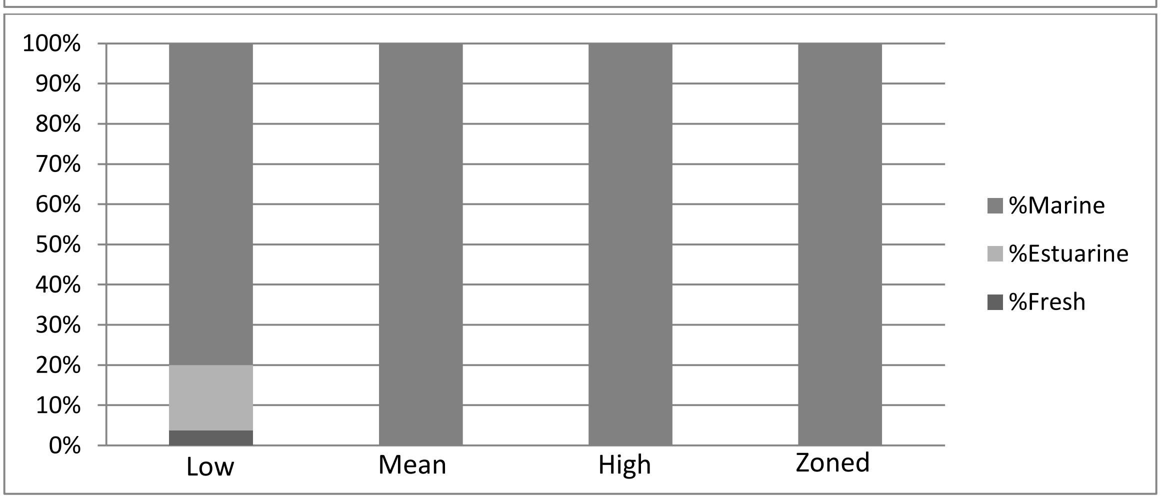
Figure AB.135. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 197. Figure 135.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

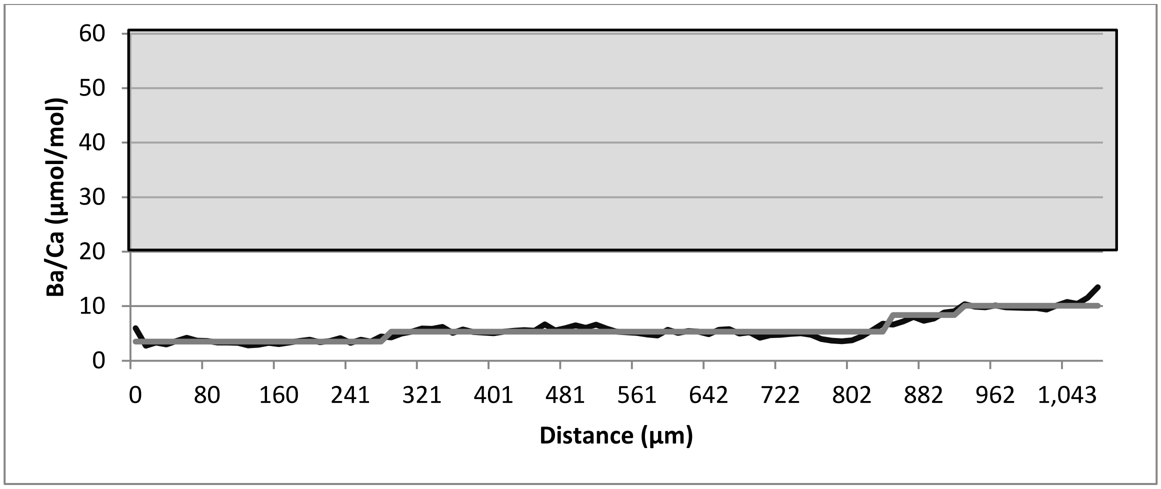


B.

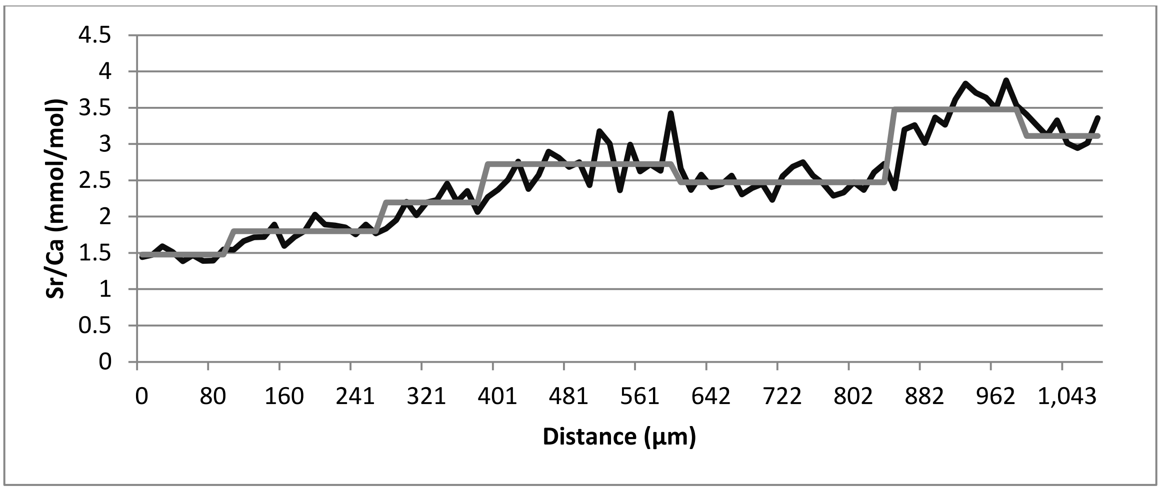


C.

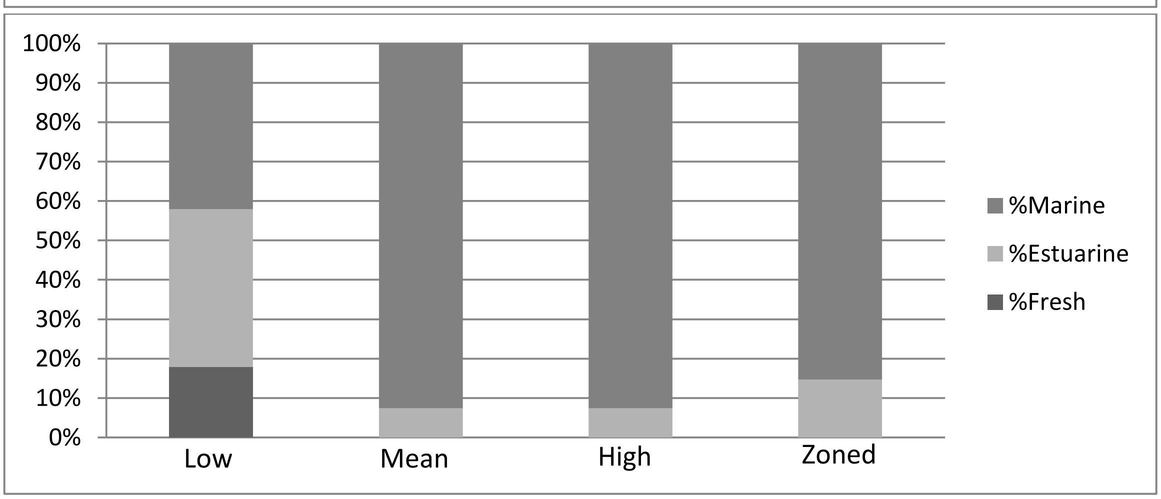
Figure AB.136. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 200. Figure 136.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

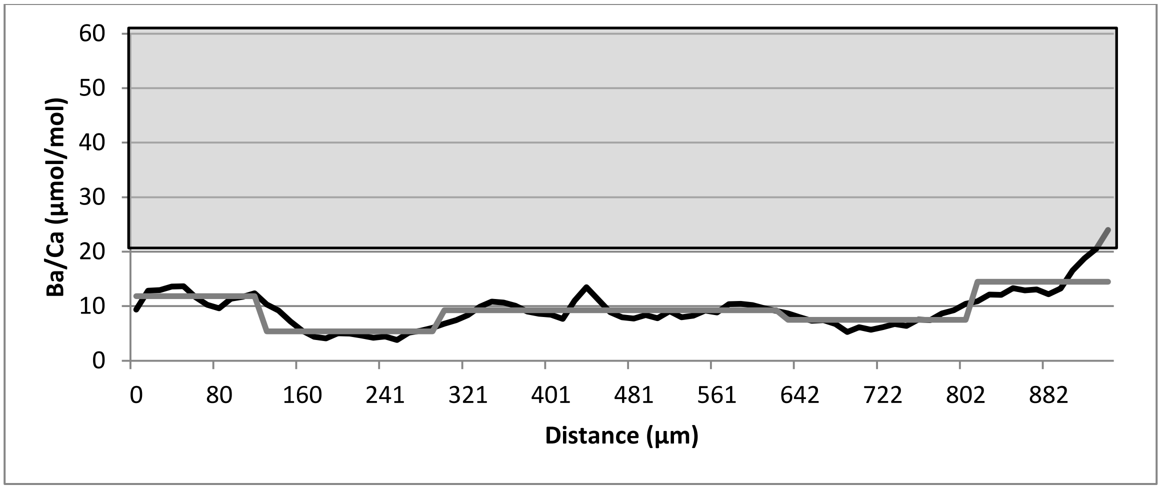


B.

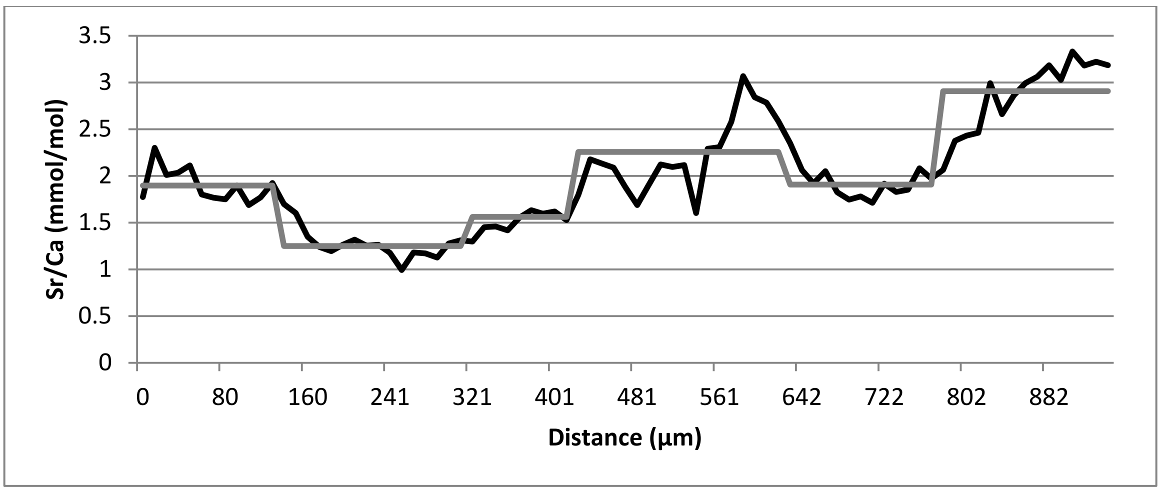


C.

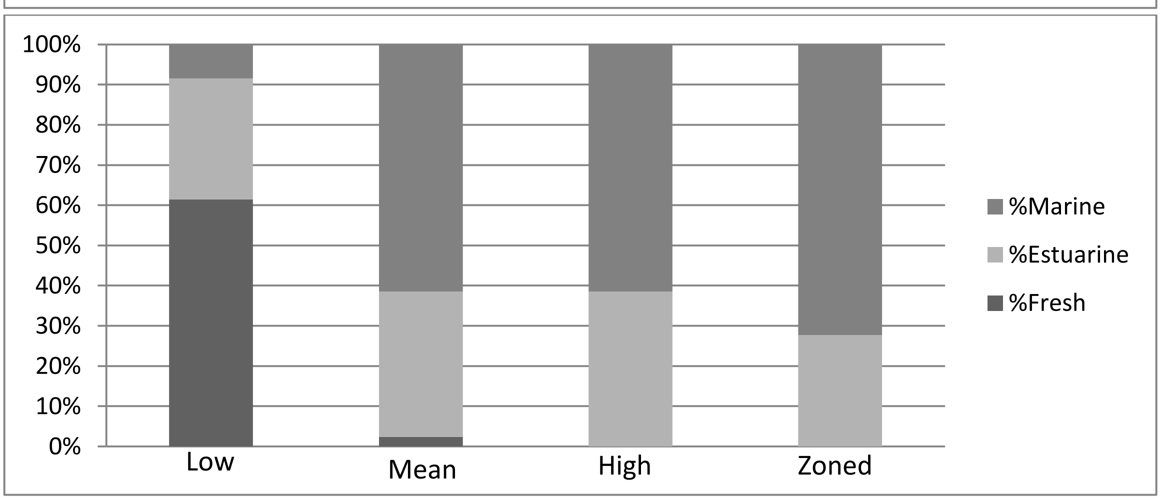
Figure AB.137. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 201. Figure 137.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.138. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 202. Figure 138.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

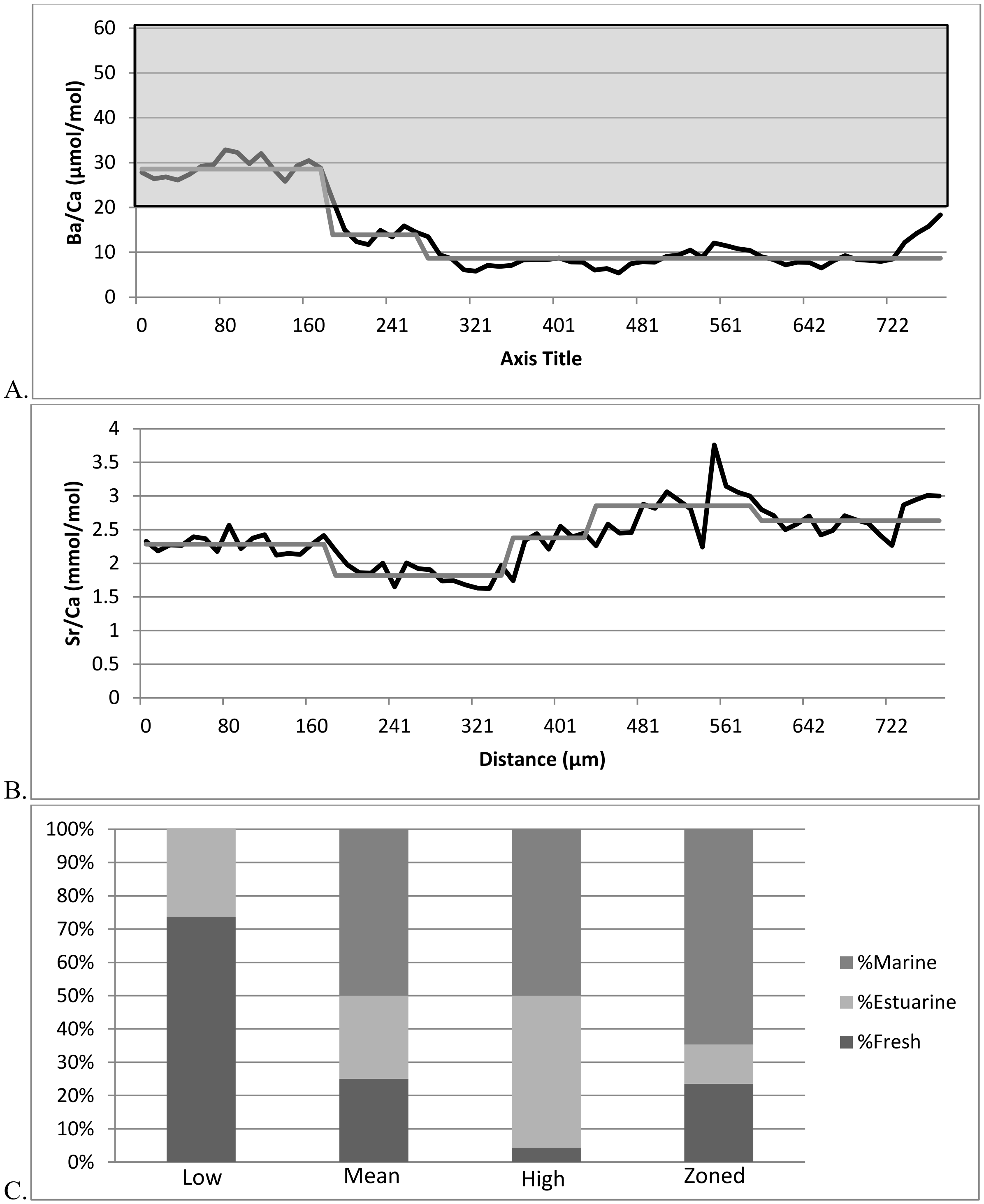
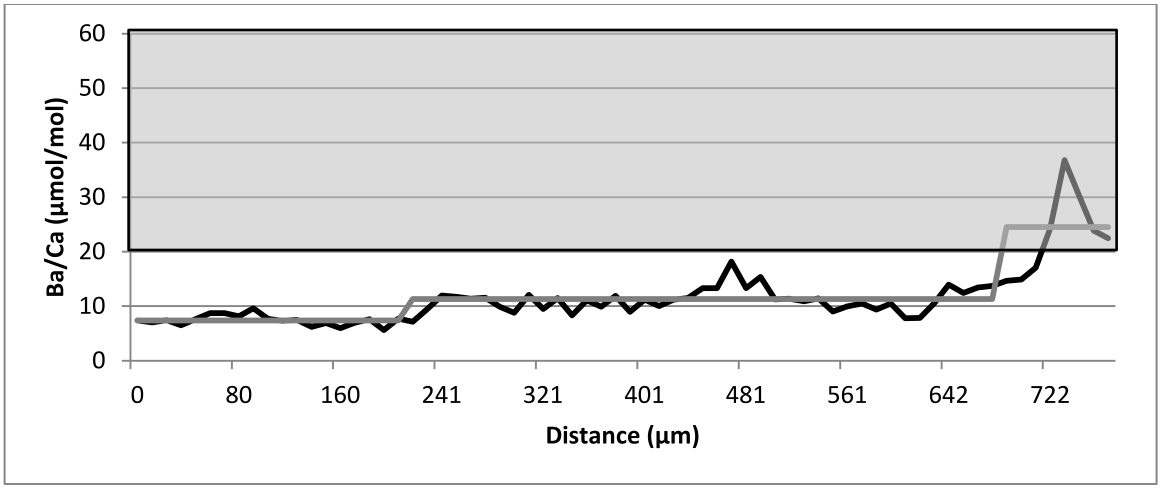
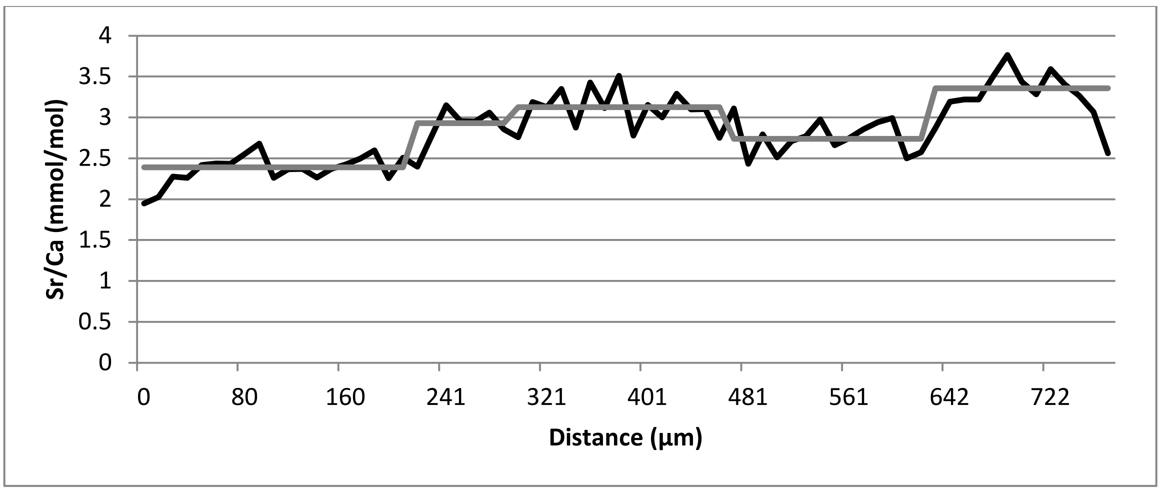


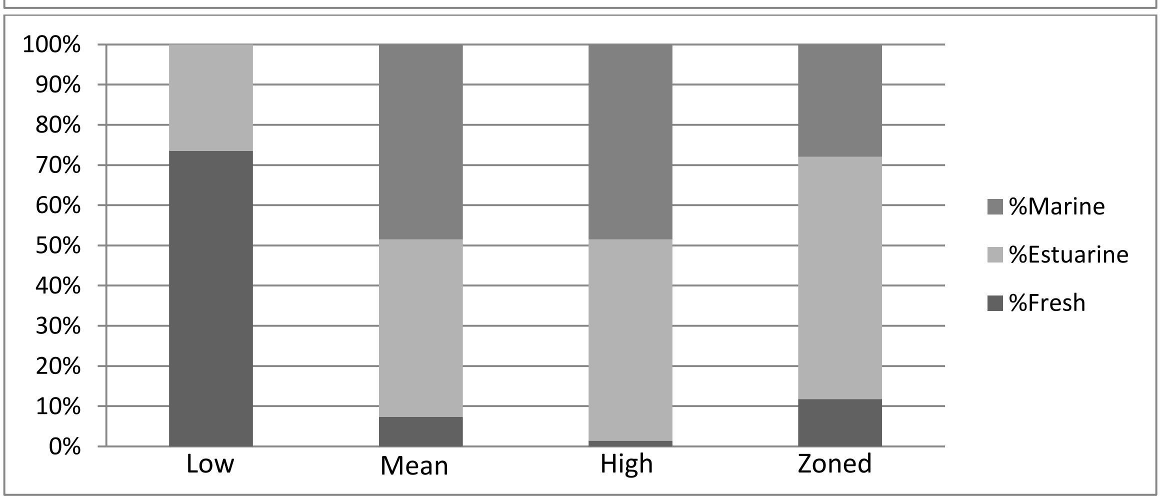
Figure AB.139. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 204. Figure 139.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

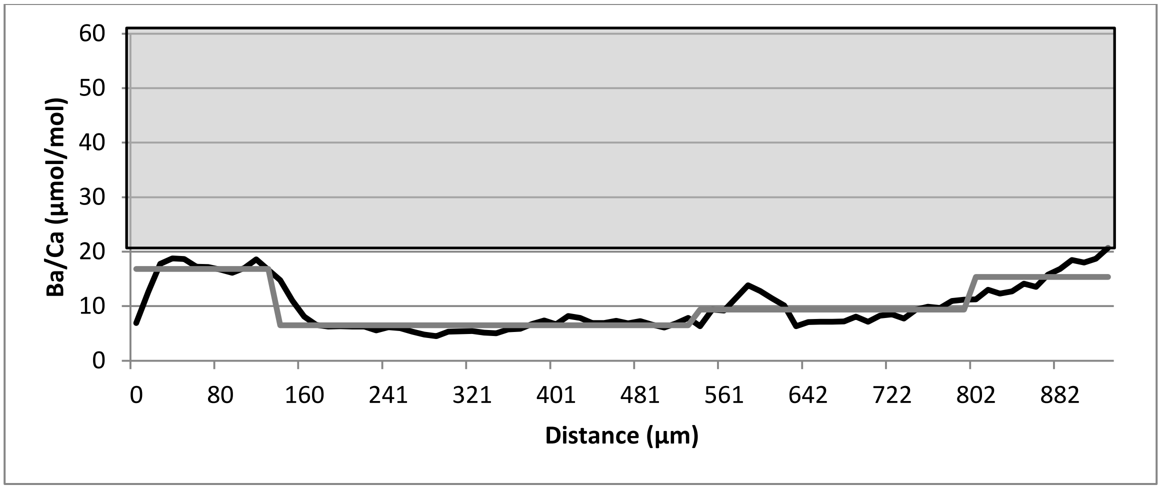


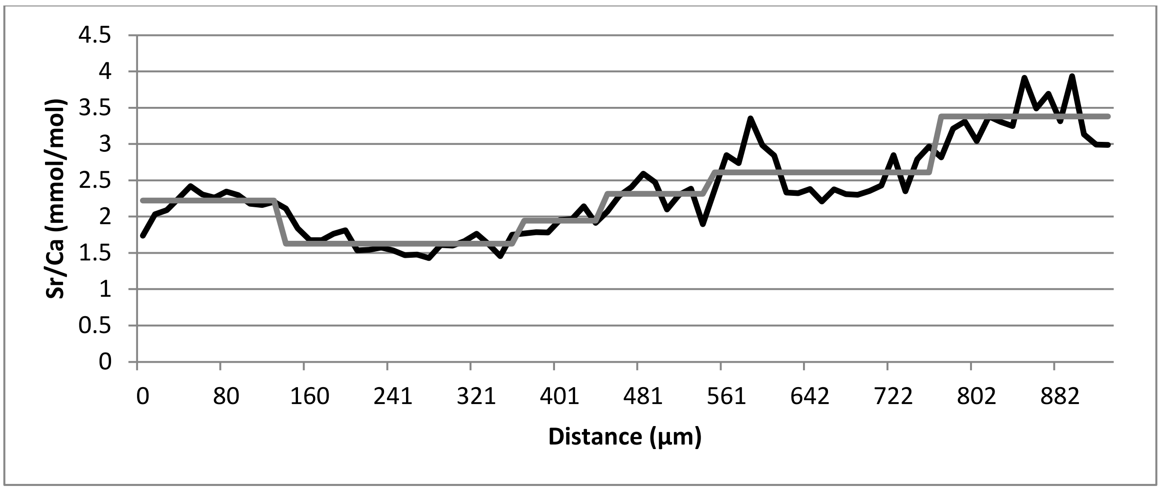
B.



C.

Figure AB.140. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 205. Figure 140.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


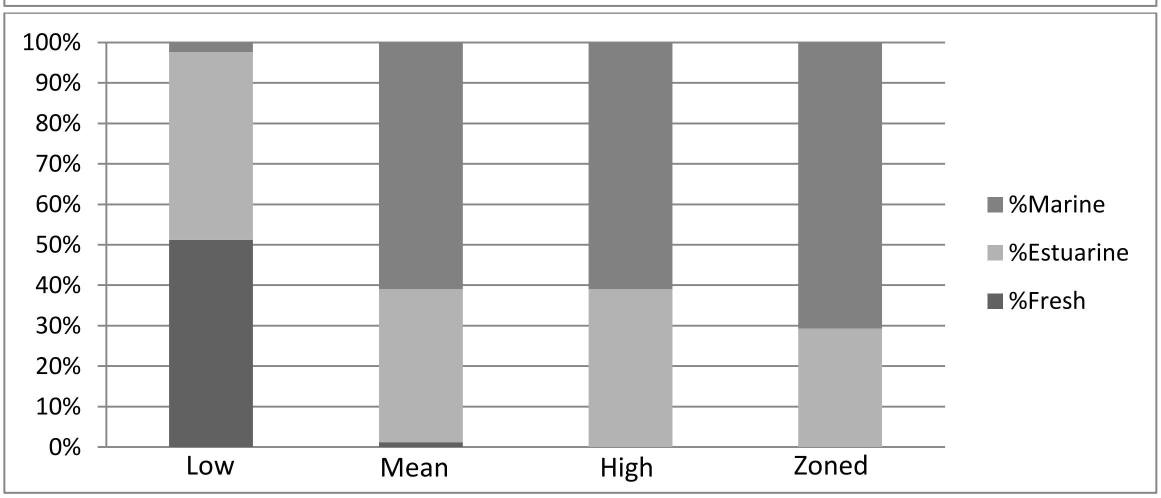
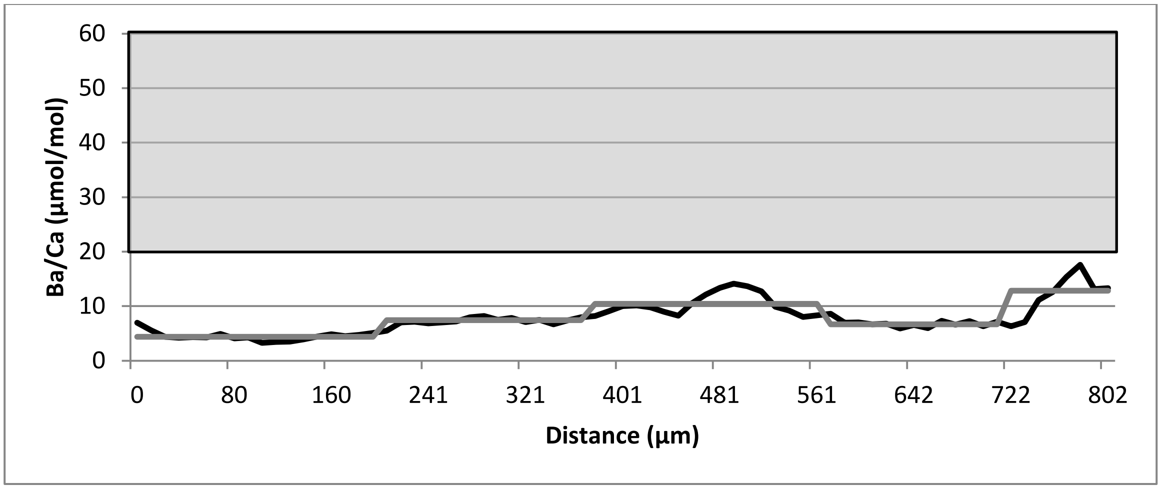
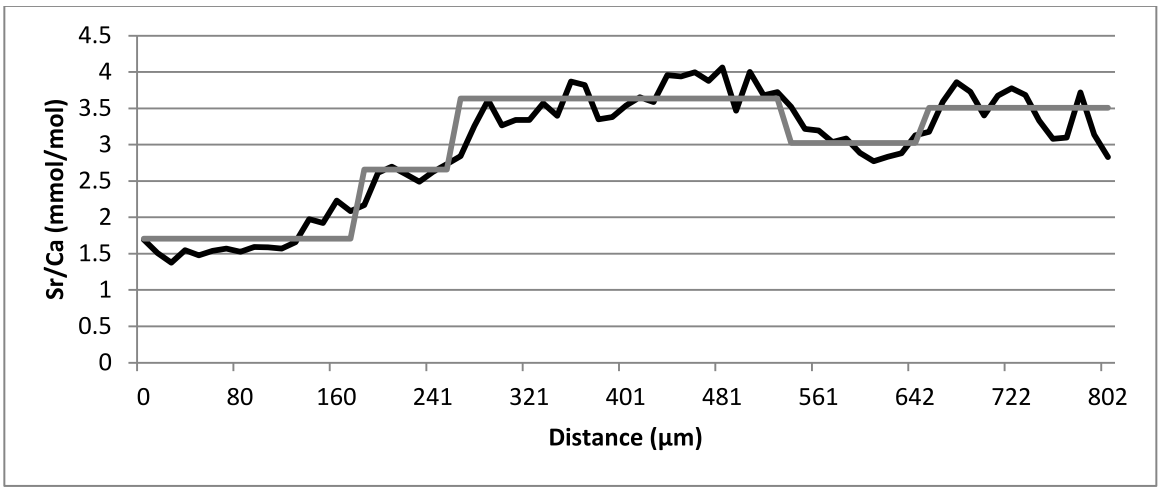
C.


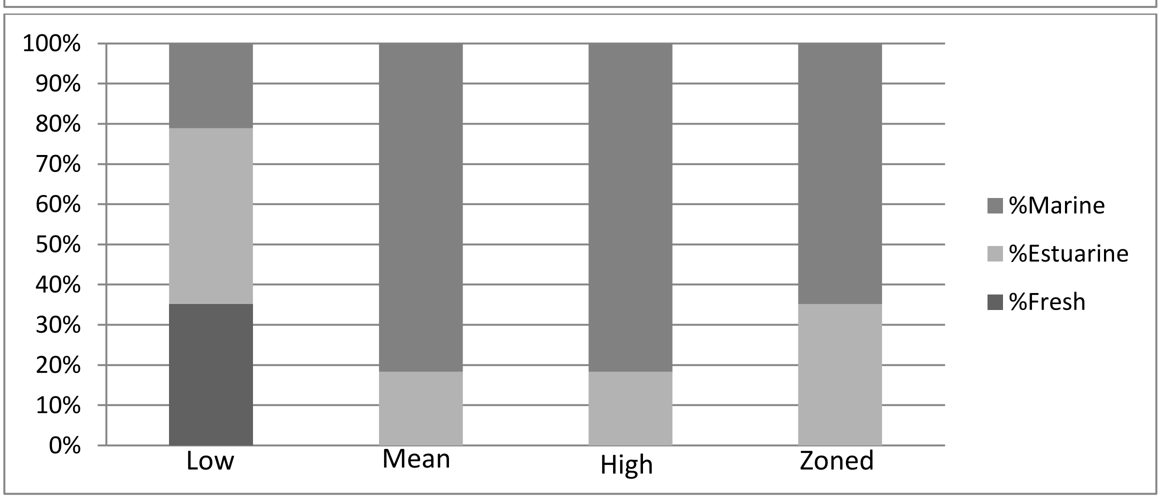
Figure AB.141. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 206. Figure 141.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

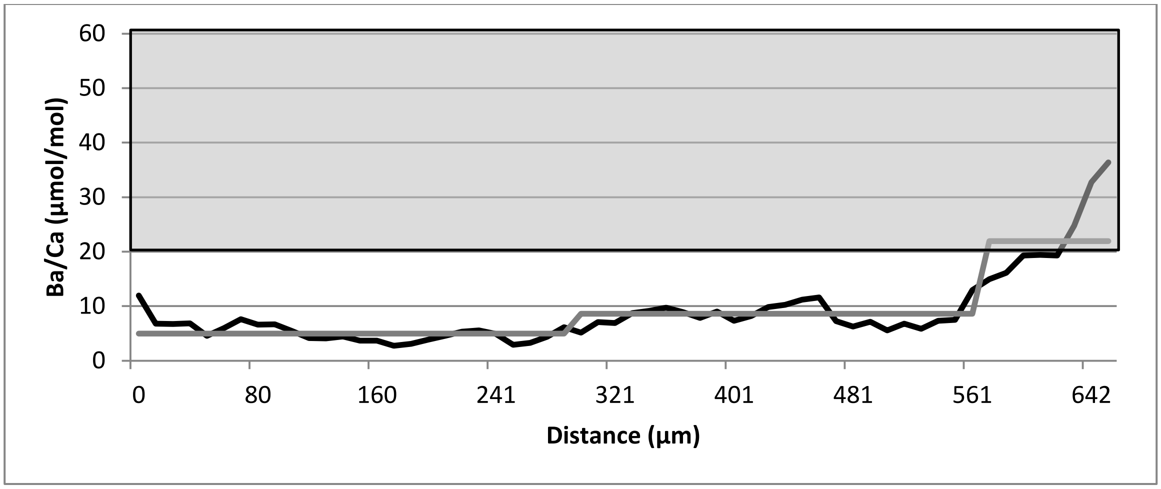


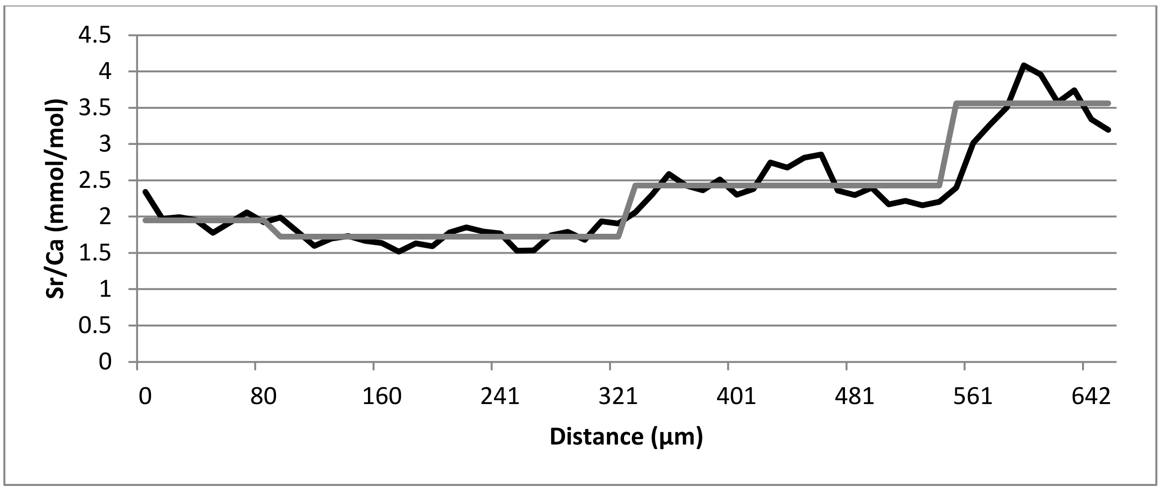
B.



C.

Figure AB.142. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 207. Figure 142.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


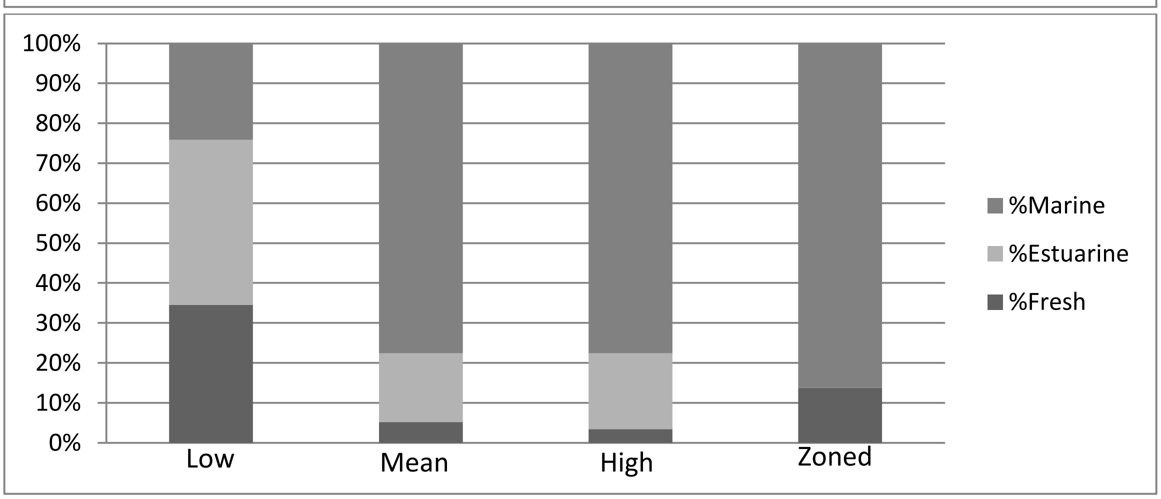
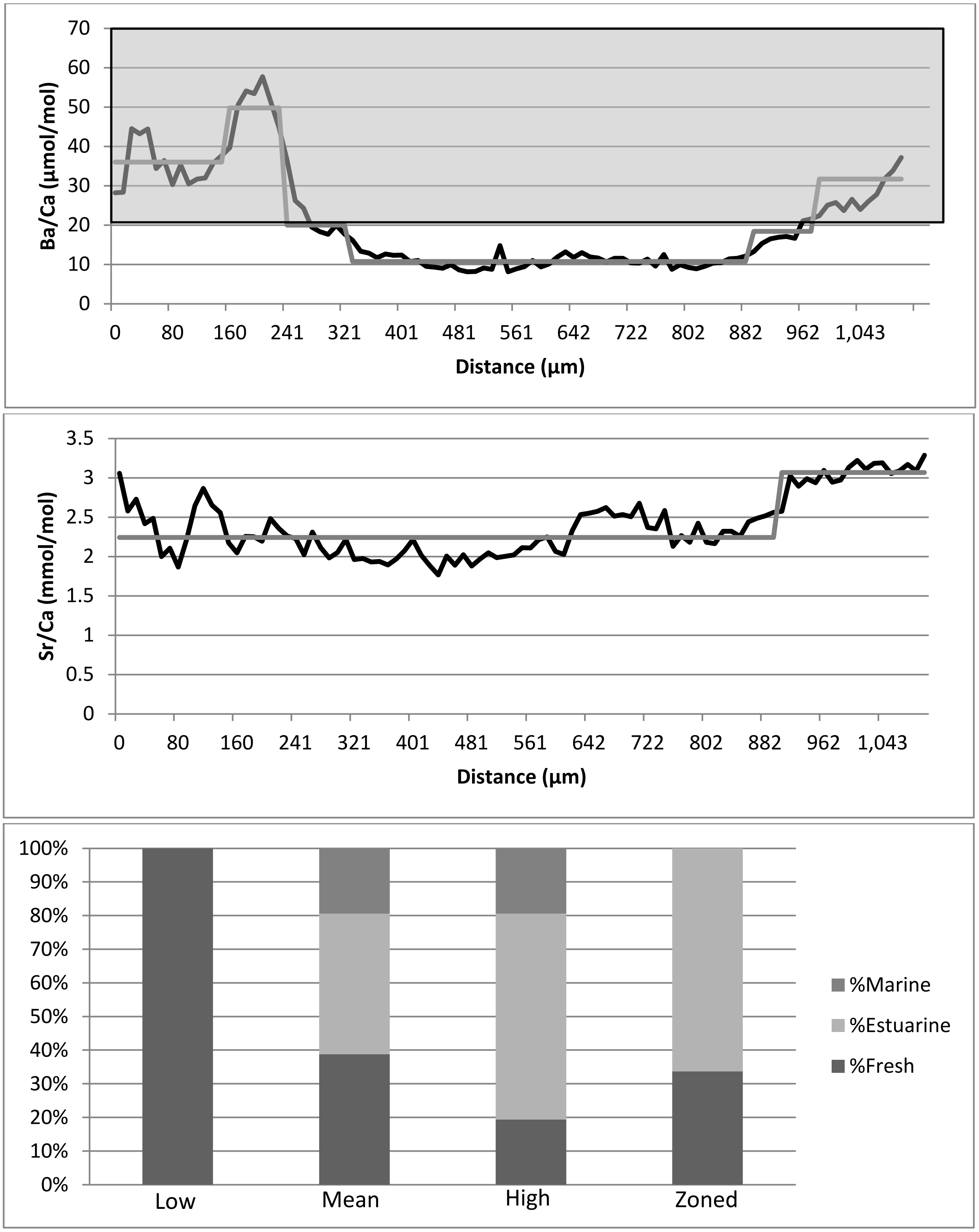
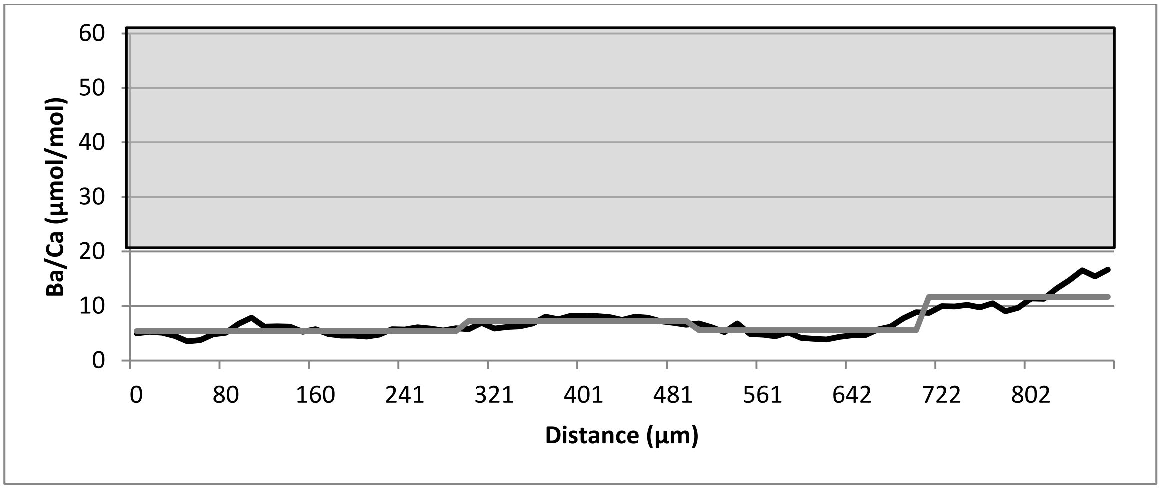
C.


Figure AB.143. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 209. Figure 143.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

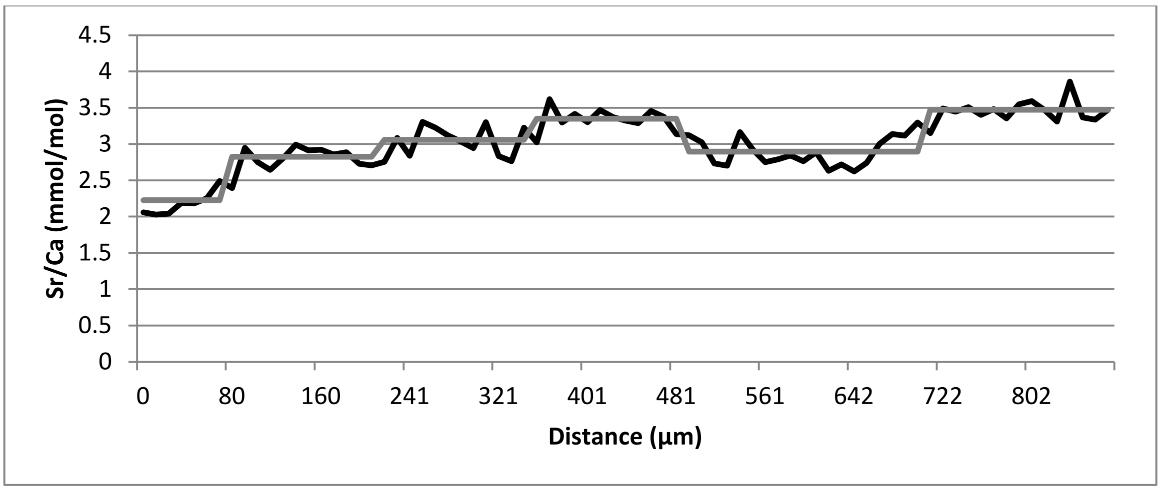


A. B. C.

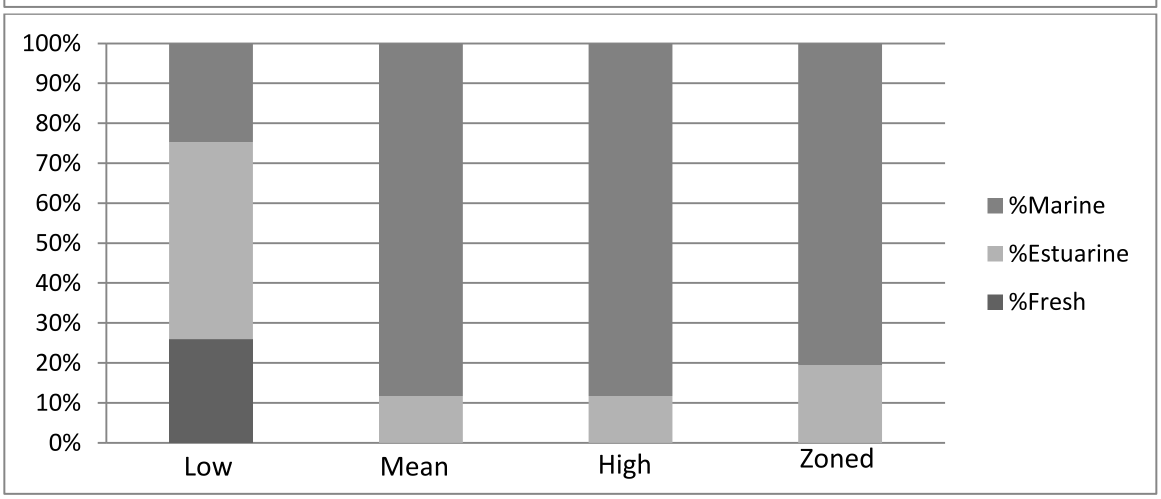
Figure AB.144. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 215. Figure 144.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

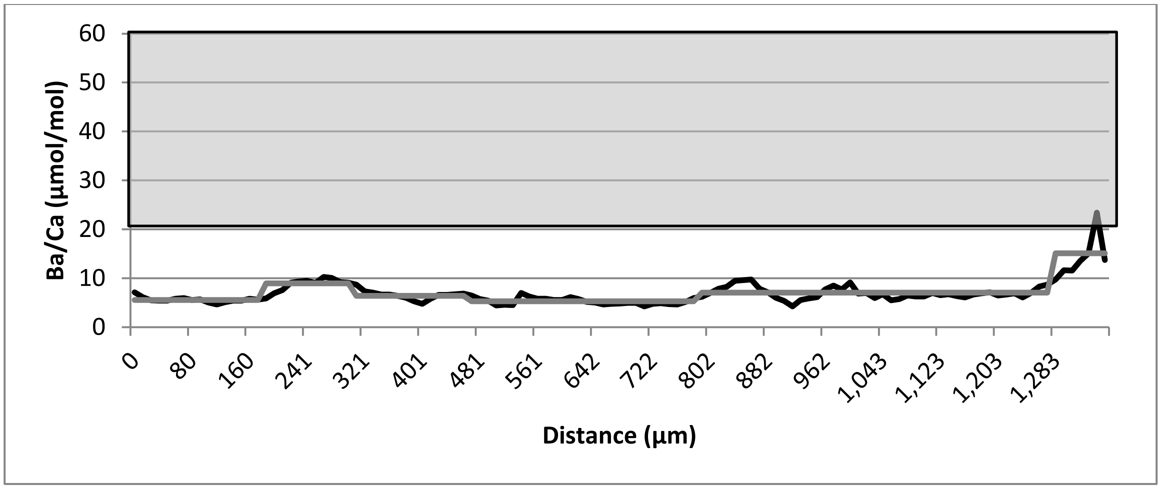


B.

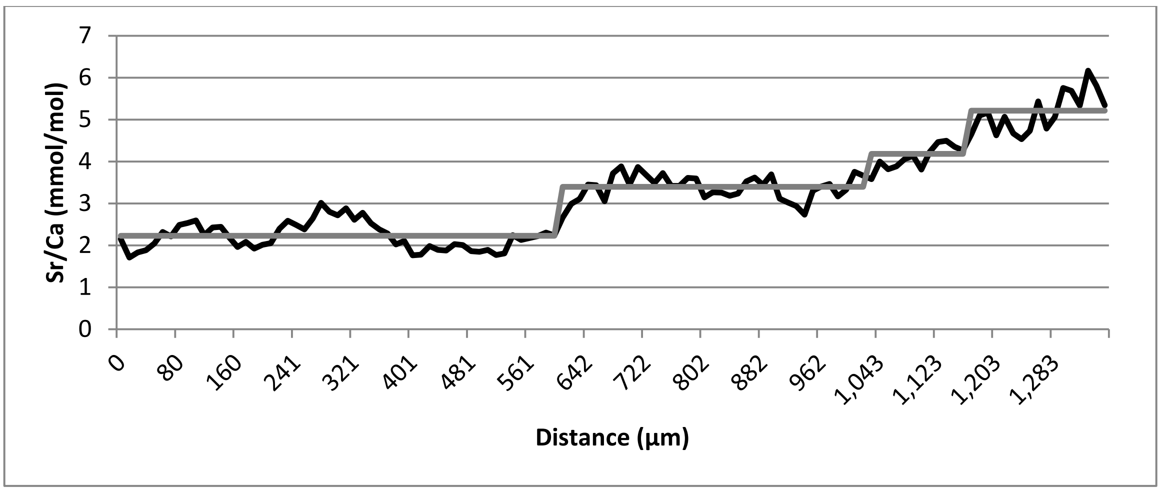


C.

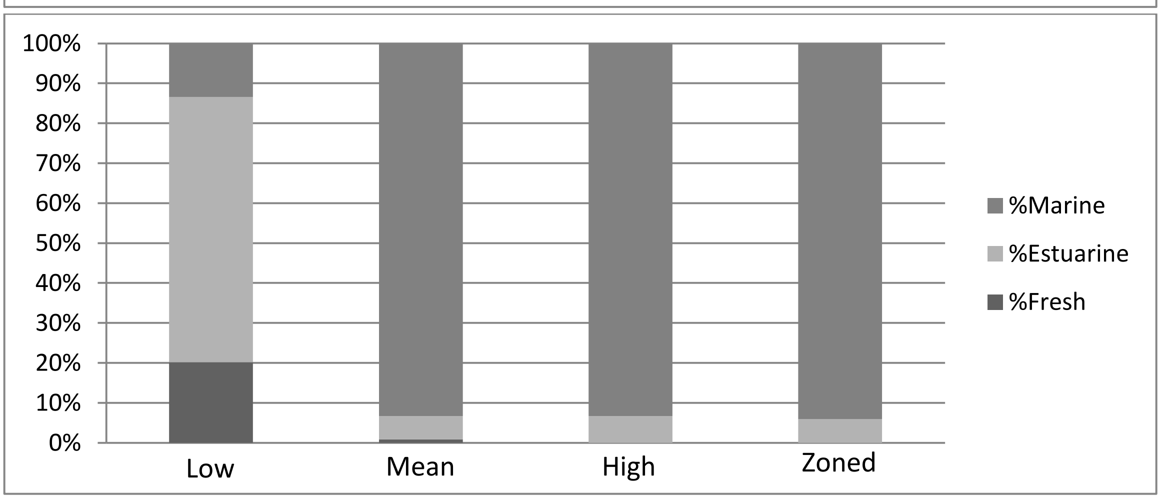
Figure AB.145. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 216. Figure 145.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

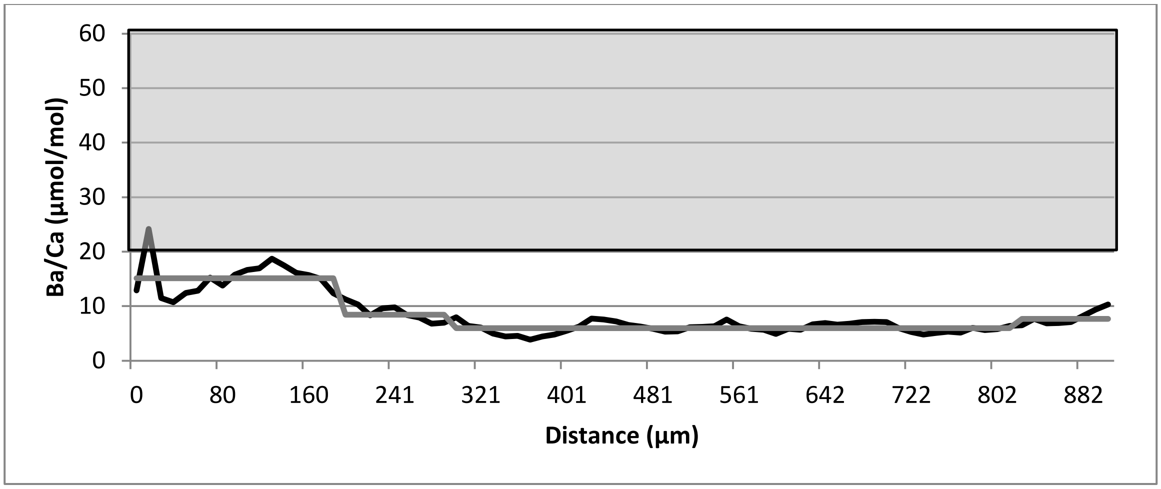


B.

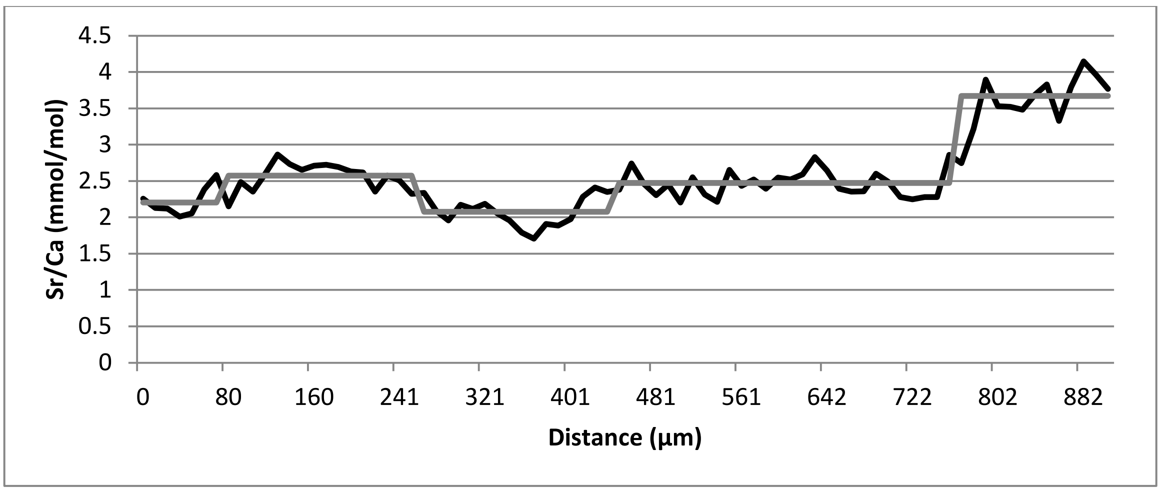


C.

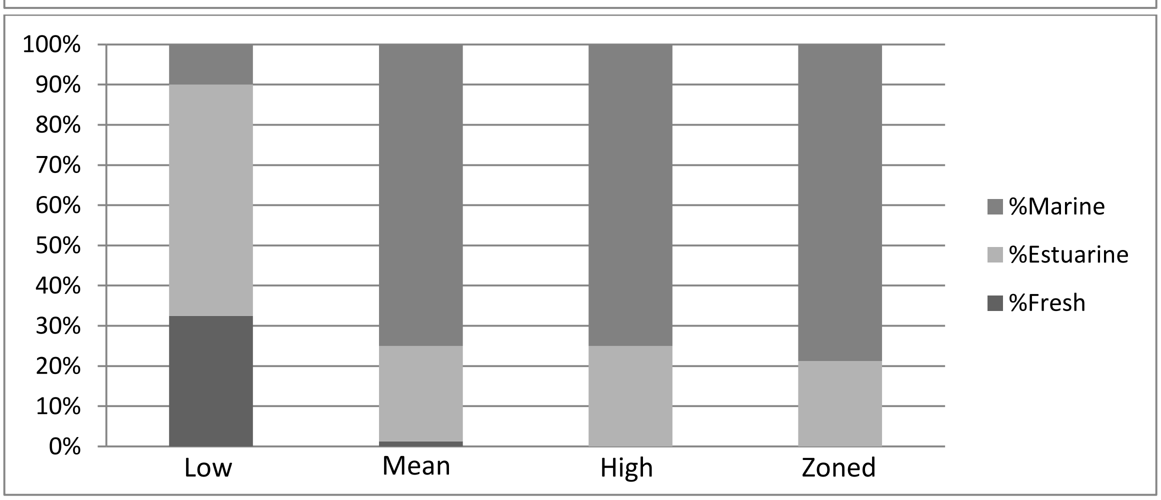
Figure AB.146. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 217. Figure 146.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

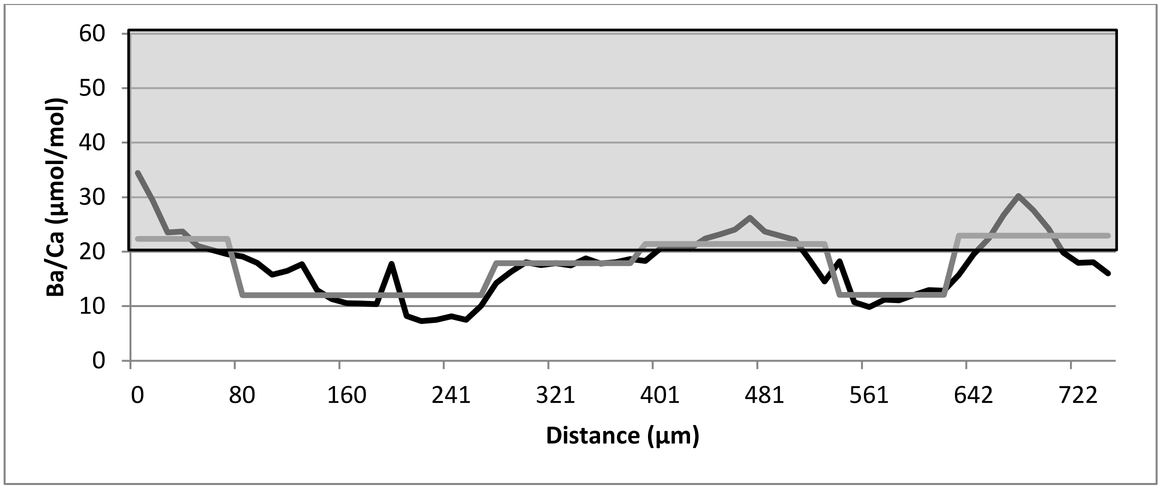


B.

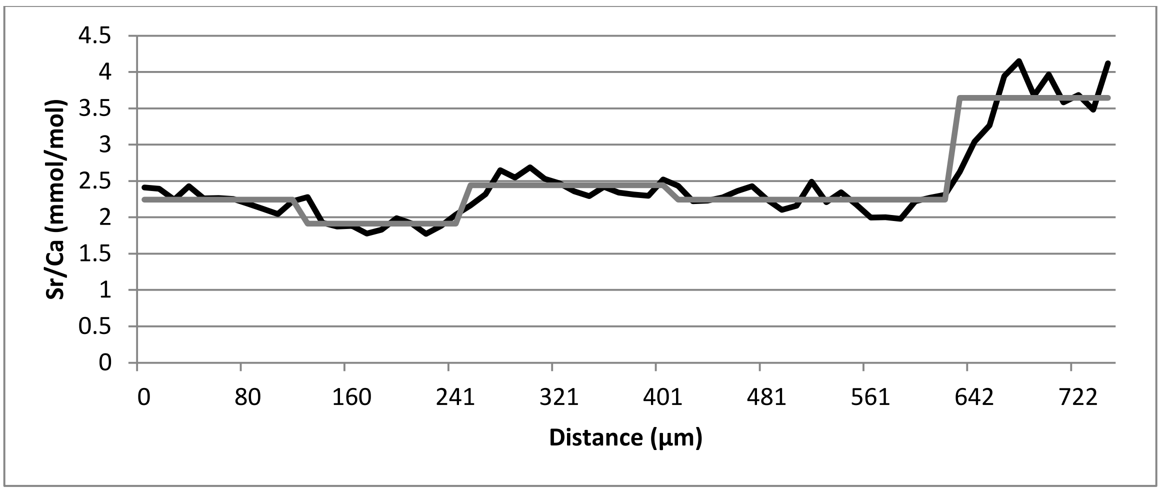


C.

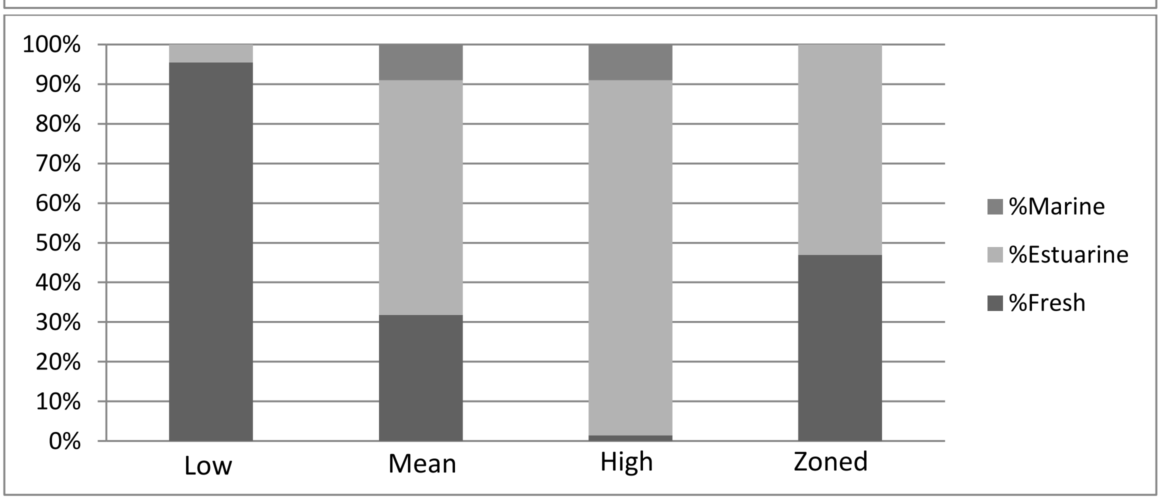
Figure AB.147. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 219. Figure 147.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

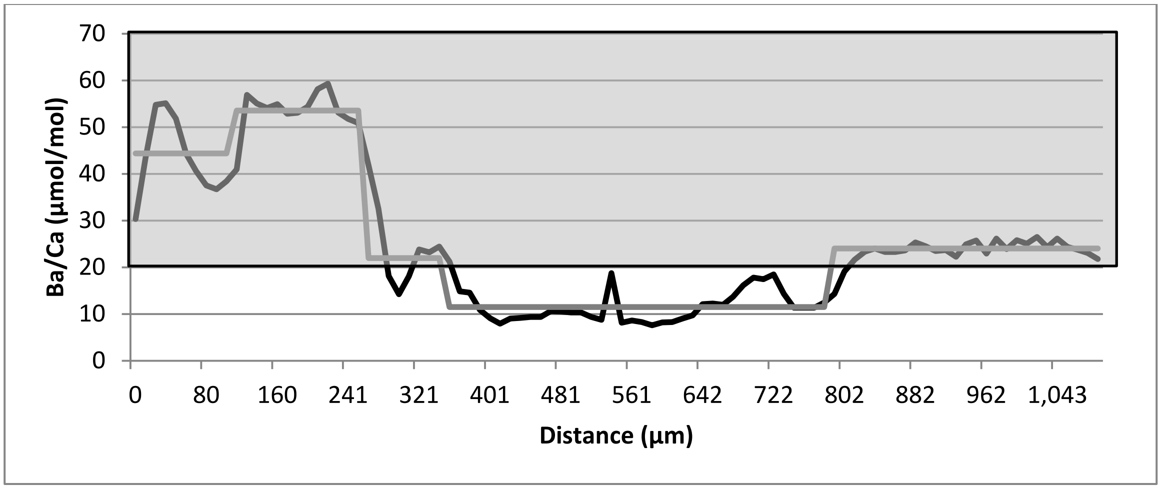


B.

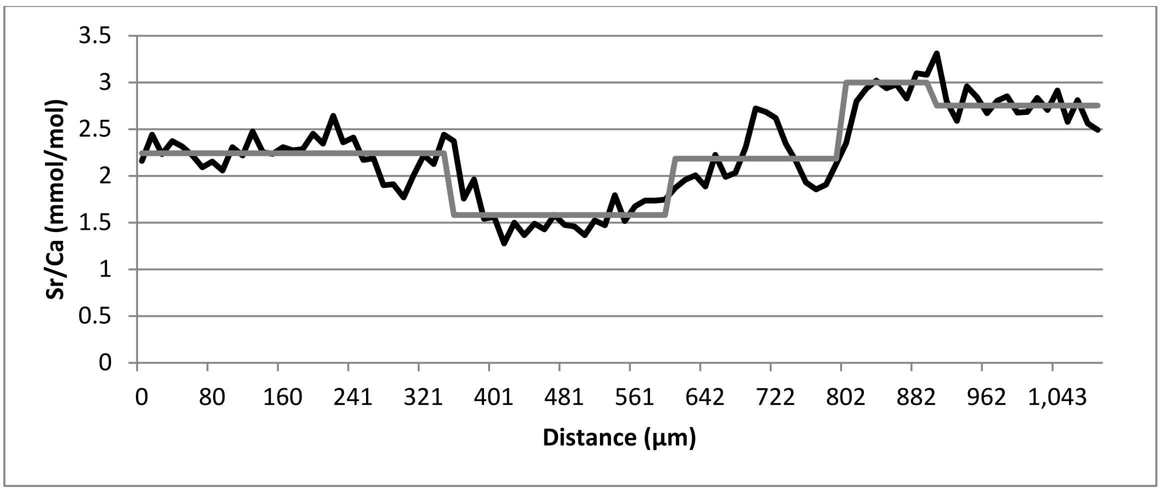


C.

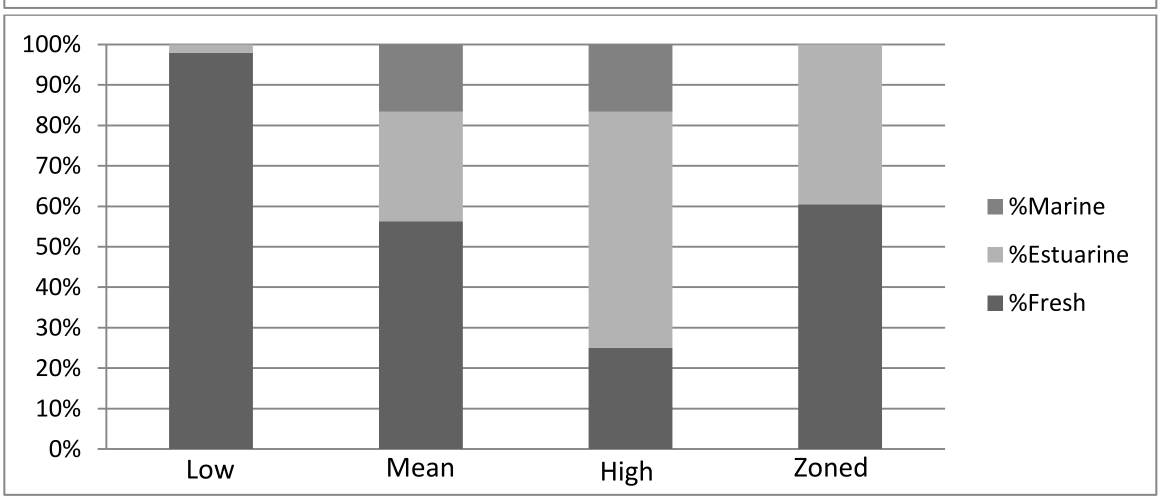
Figure AB.148. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 220. Figure 148.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

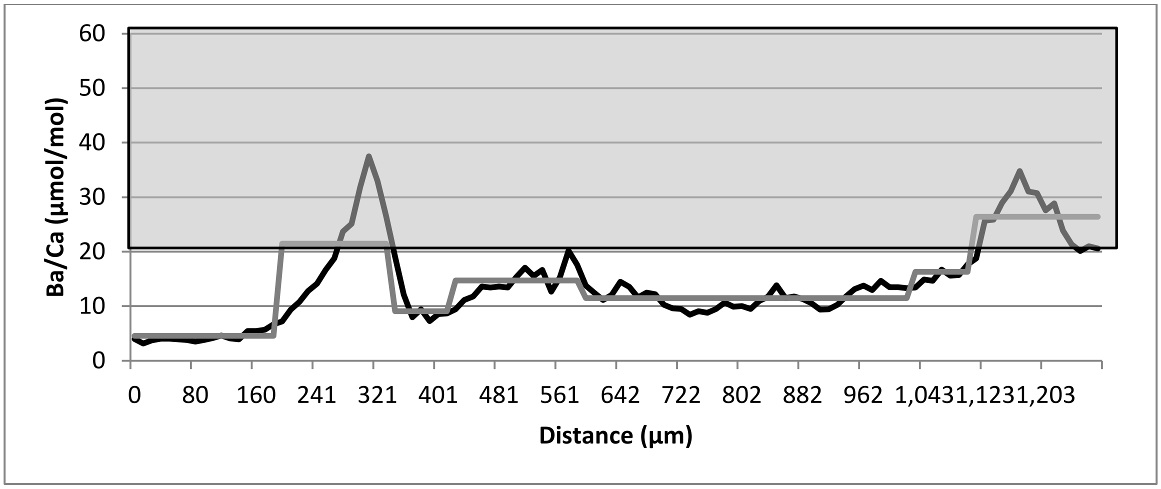


B.

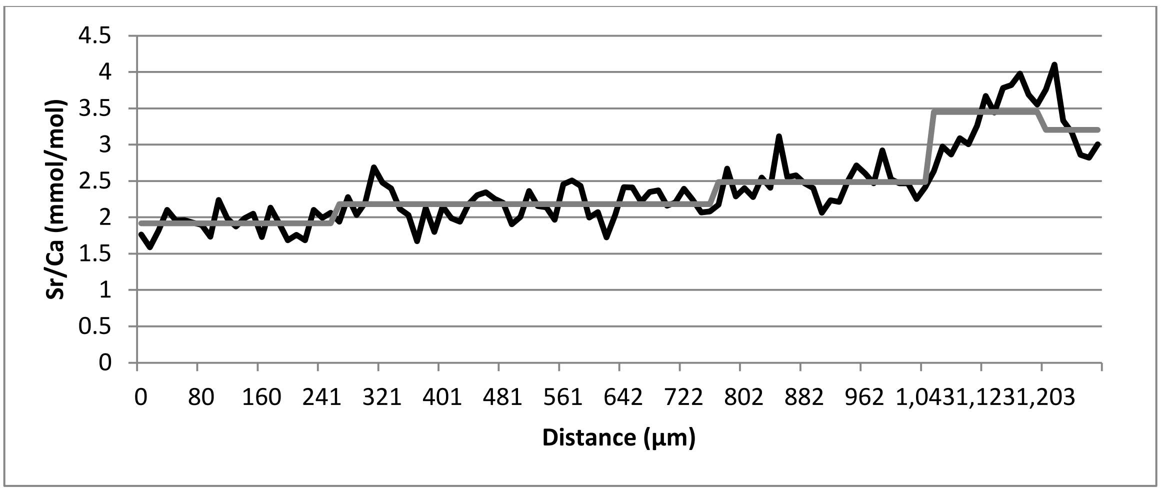


C.

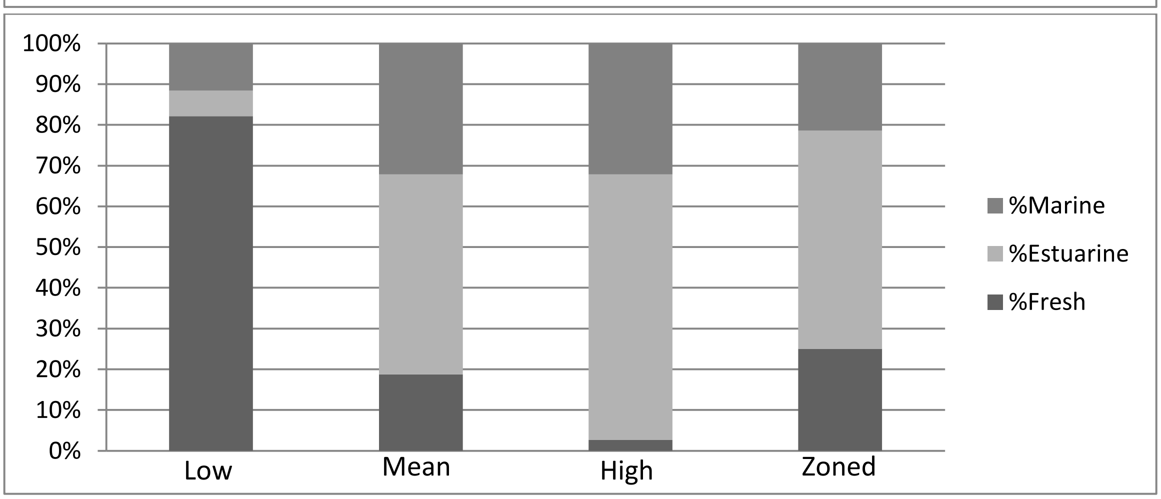
Figure AB.149. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 221. Figure 149.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.150. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 222. Figure 150.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

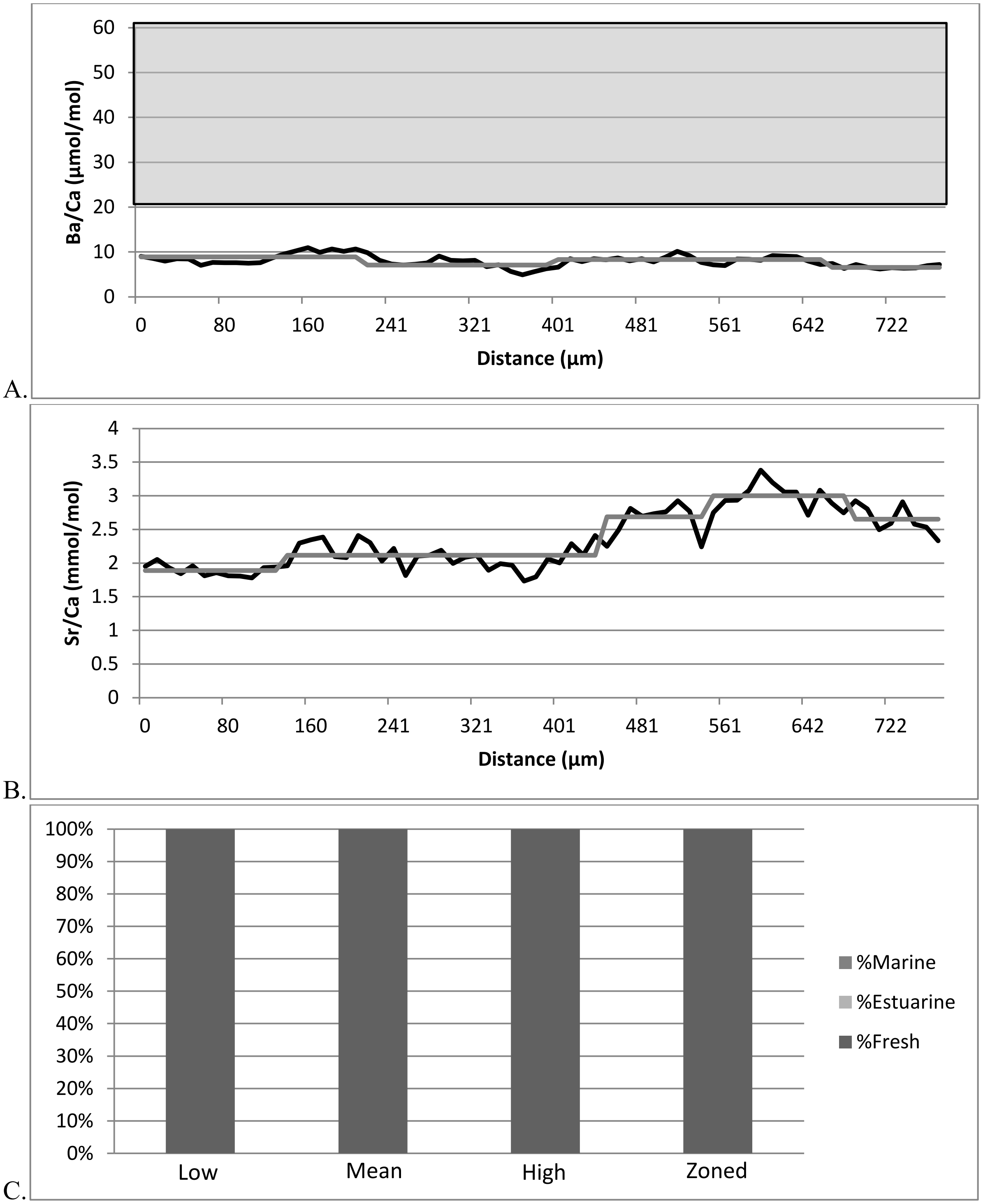


Figure AB.151. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 223. Figure 151.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

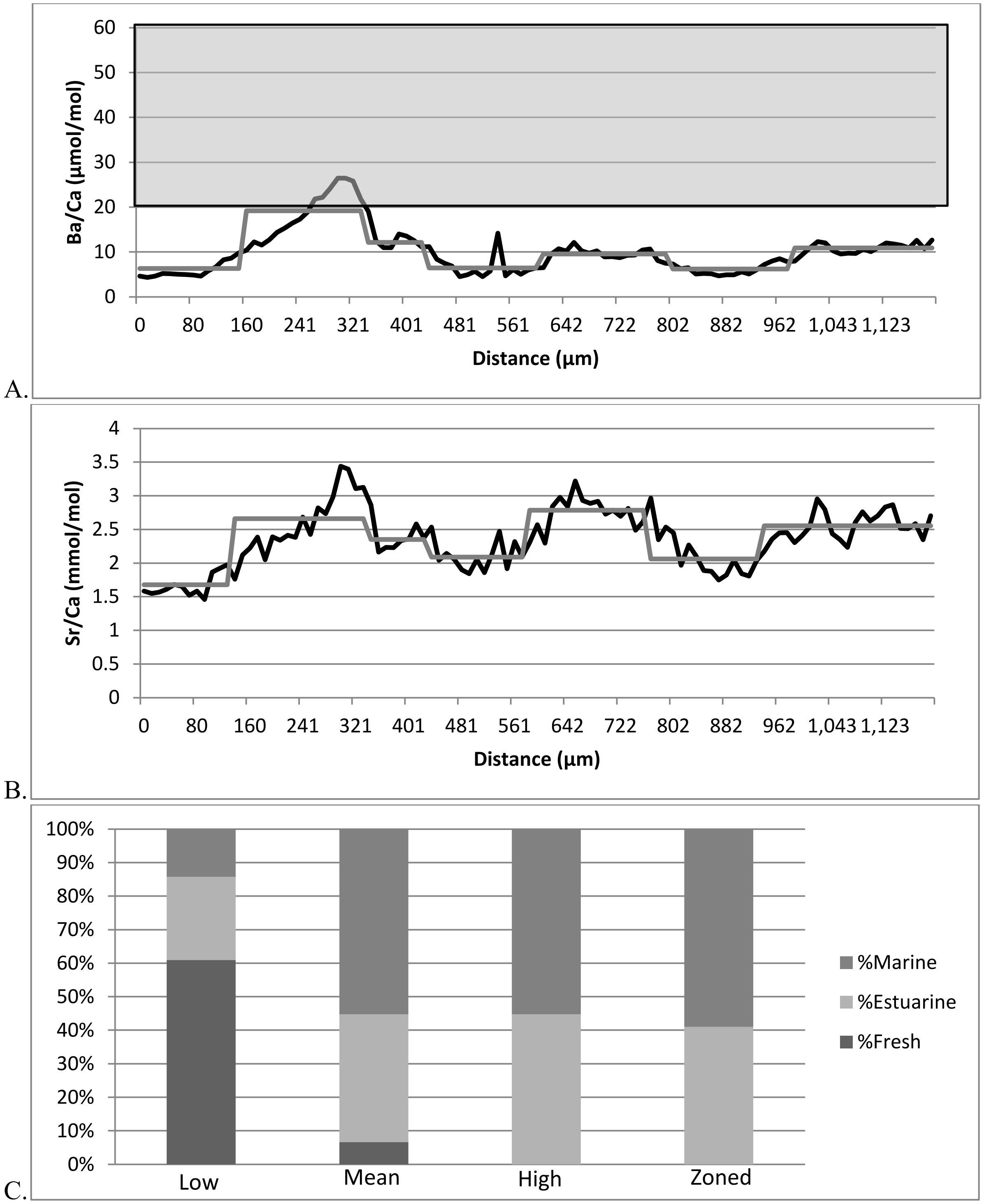


Figure AB.152. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 224. Figure 152.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

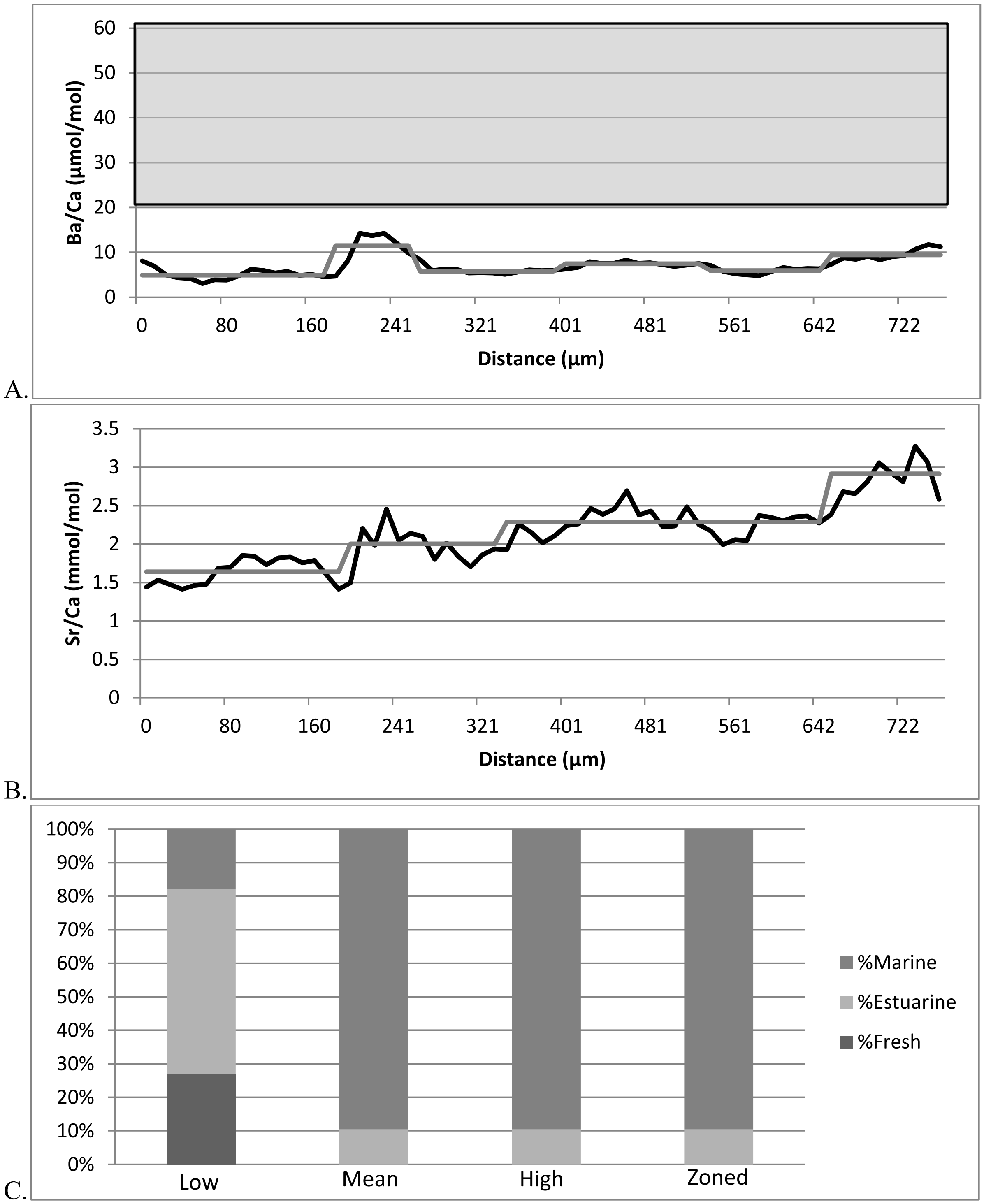
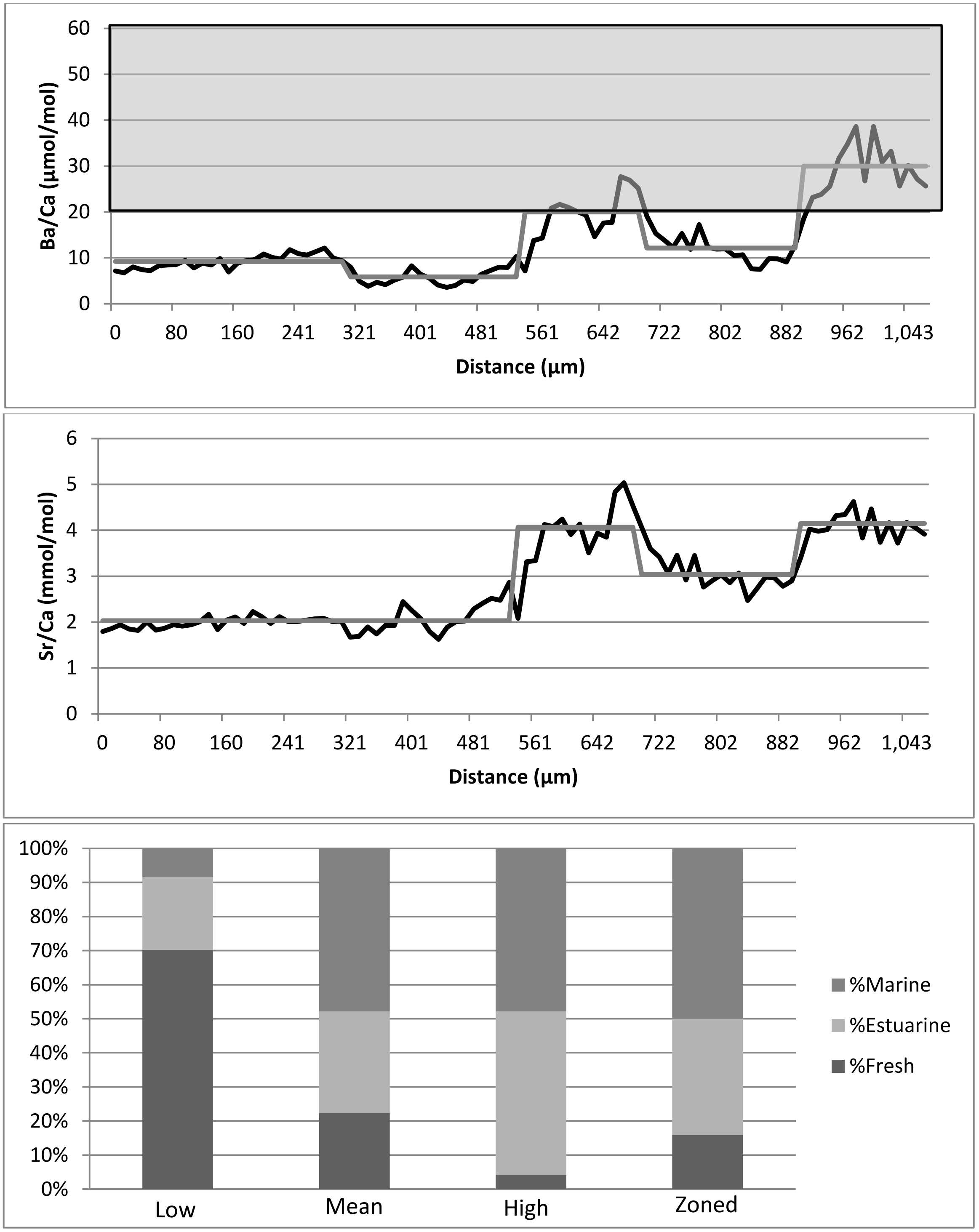


Figure AB.153. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 225. Figure 153.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

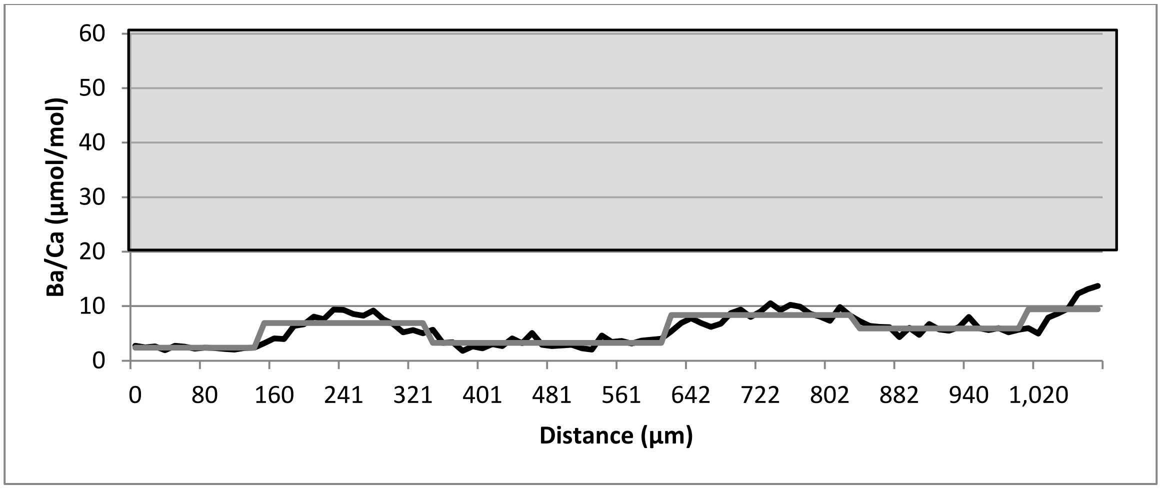
A.



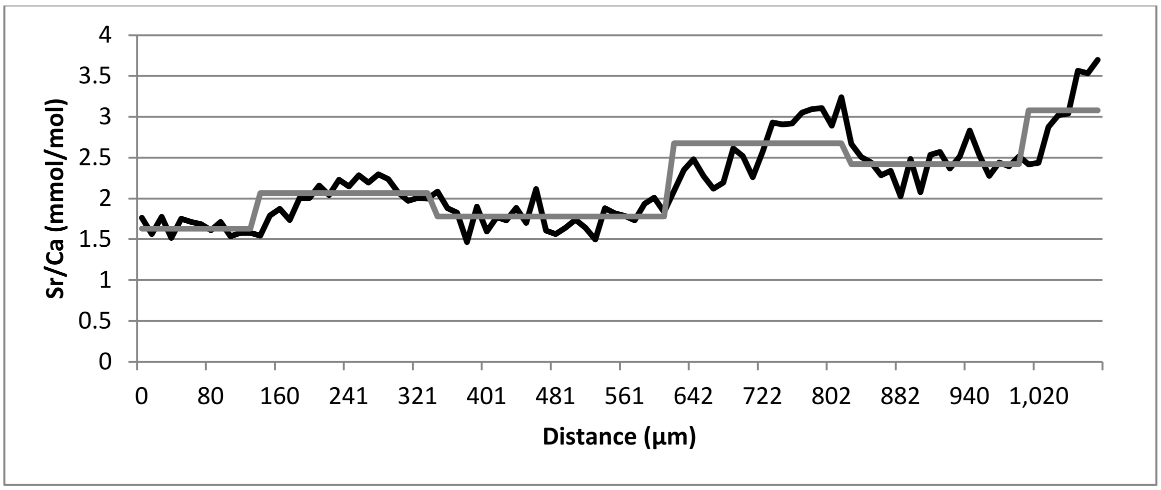
B.

C.

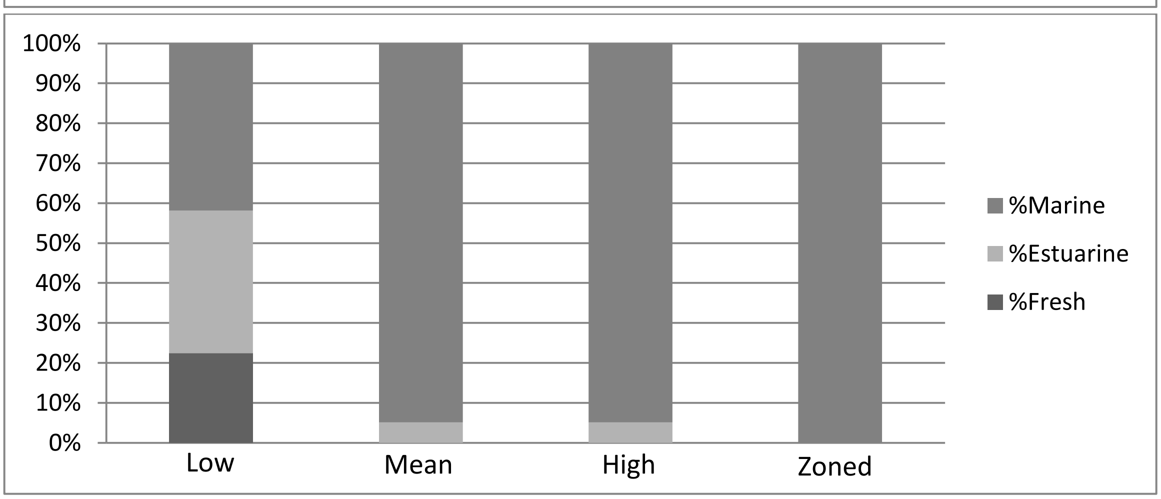
Figure AB.154. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 226. Figure 154.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.155. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 227. Figure 155.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

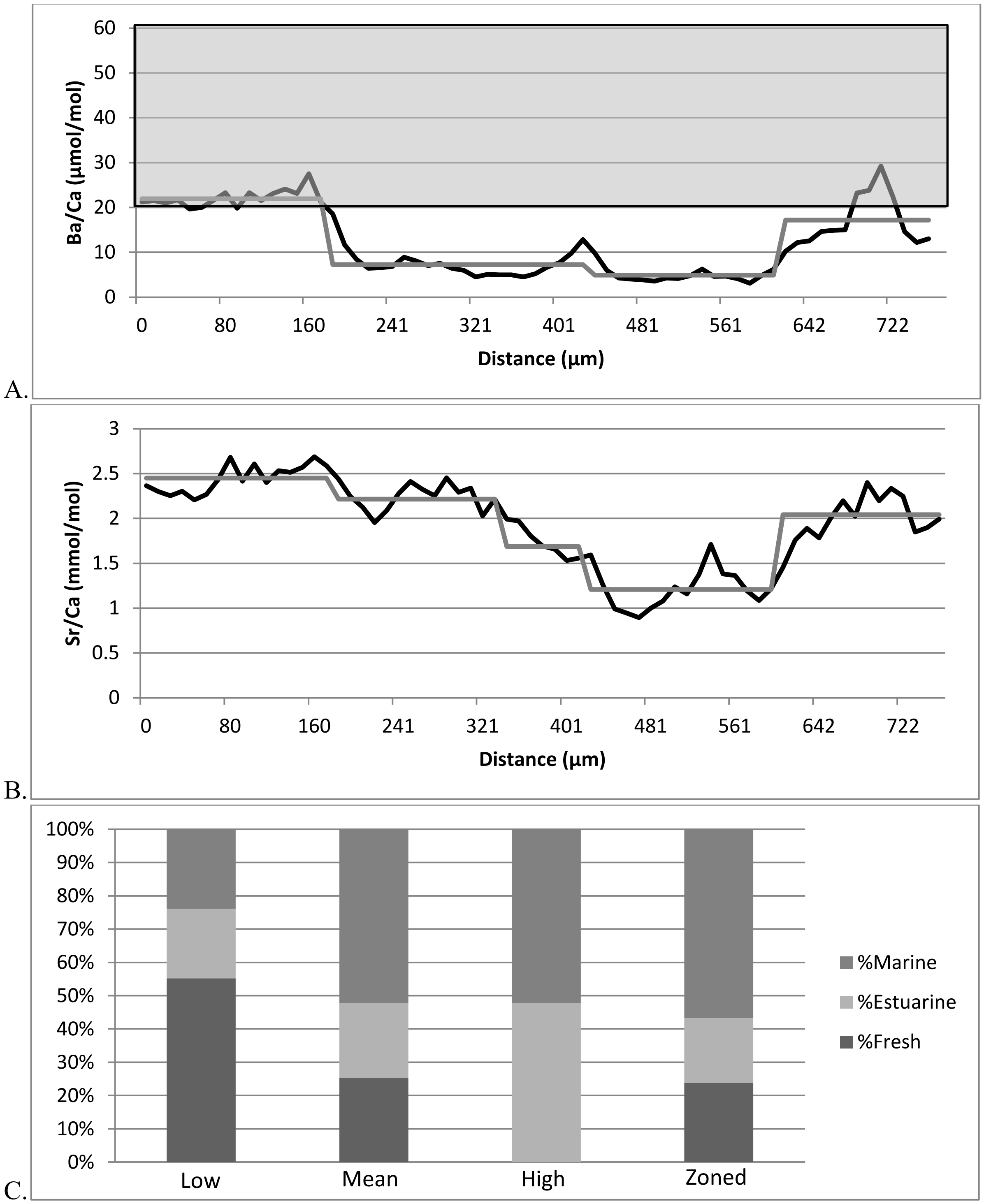
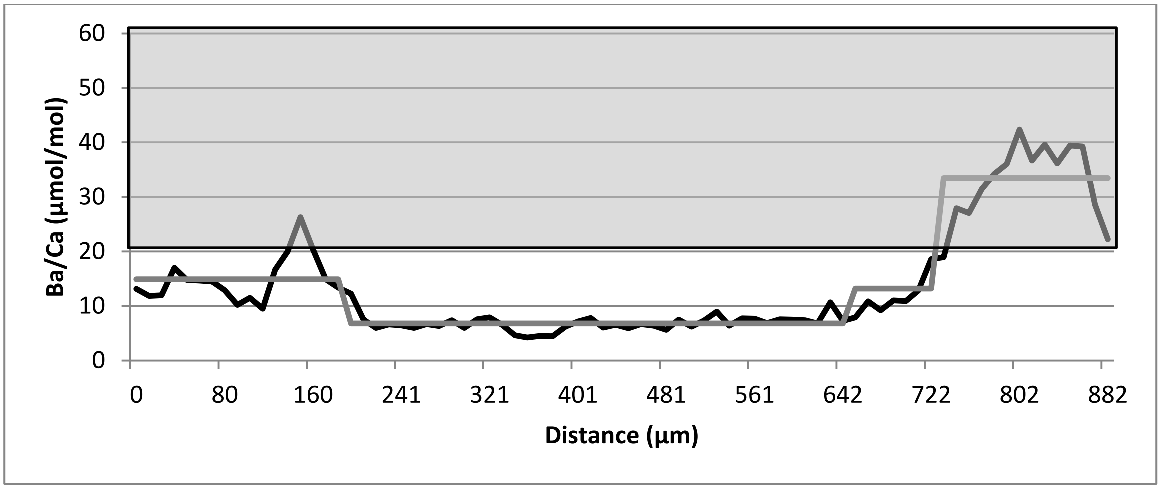
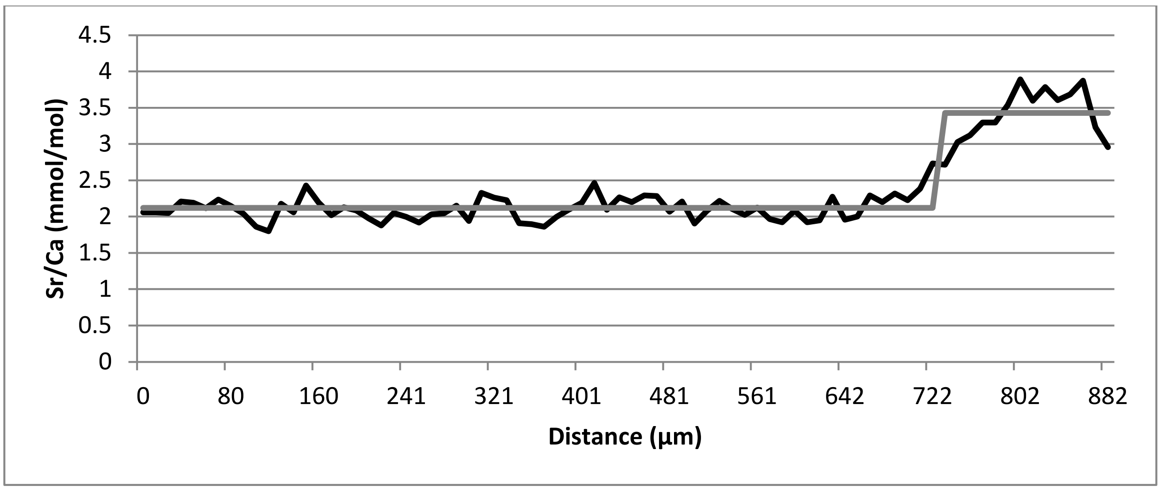


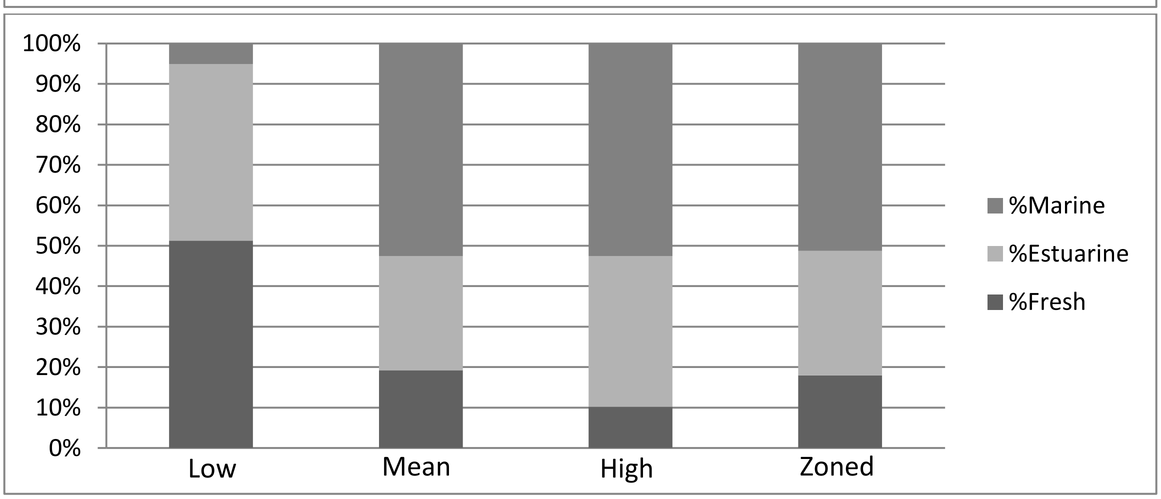
Figure AB.156. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 229. Figure 156.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.157. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 230. Figure 157.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

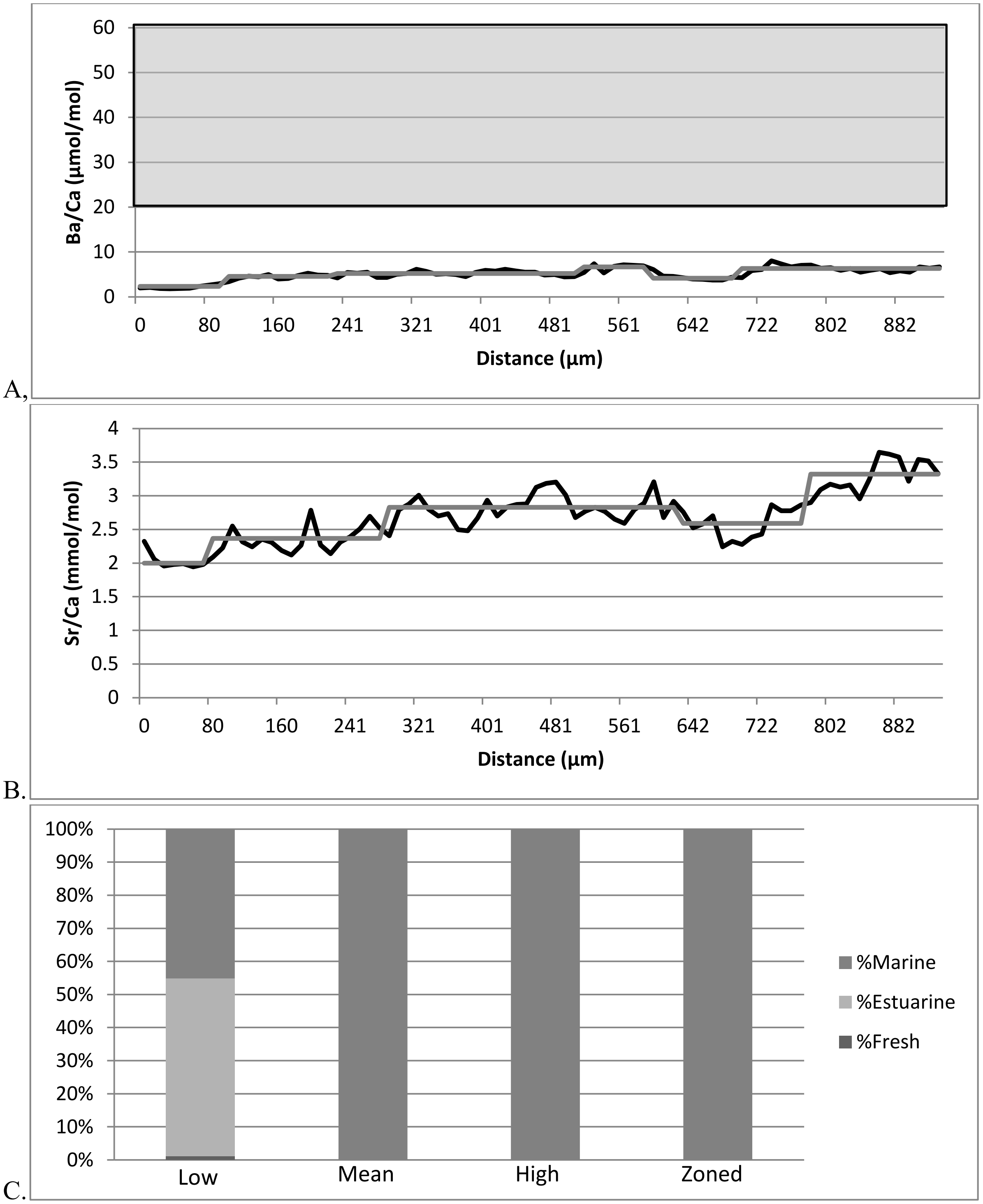


Figure AB.158. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 231. Figure 158.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

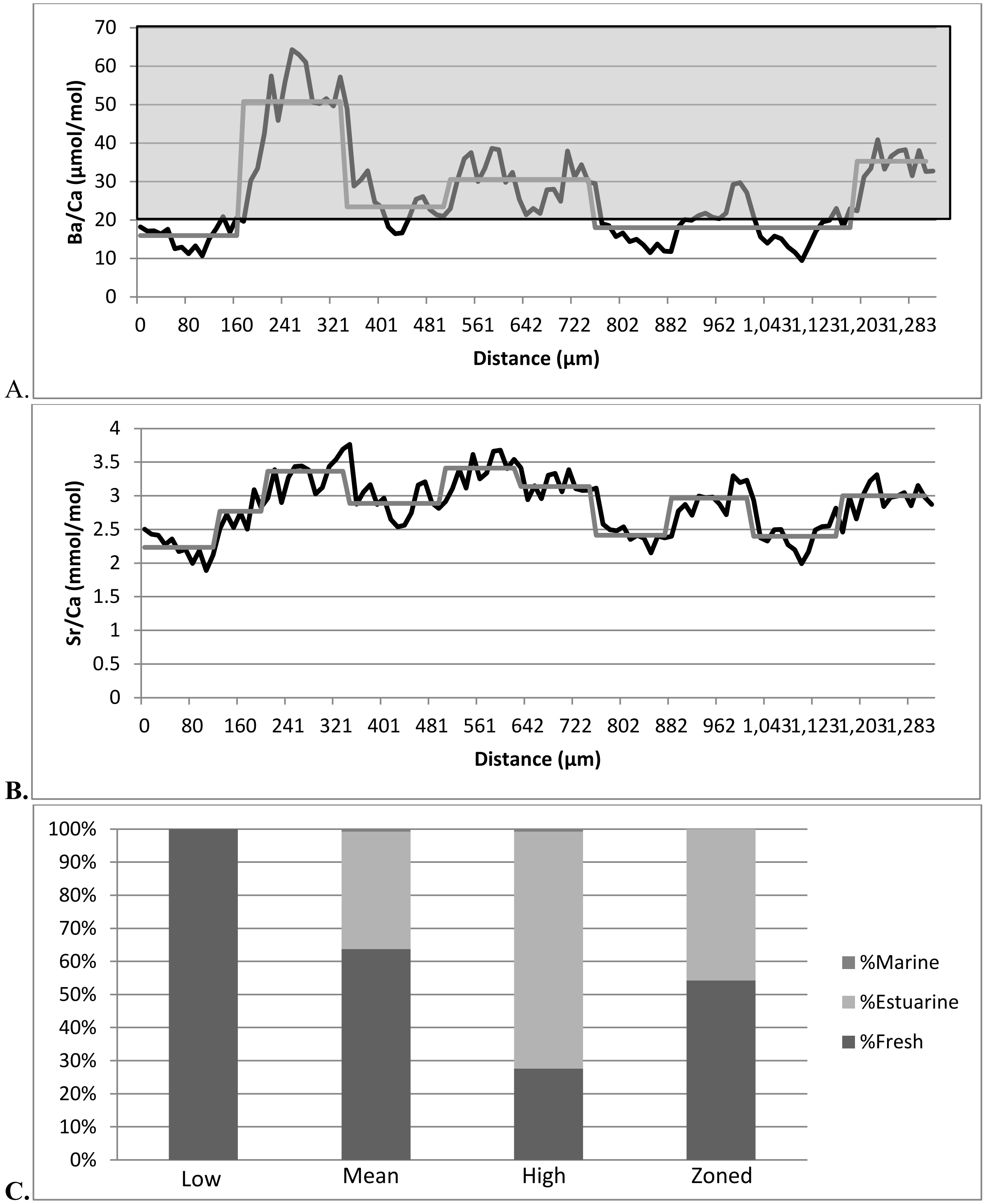


Figure AB.159. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 233. Figure 159.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

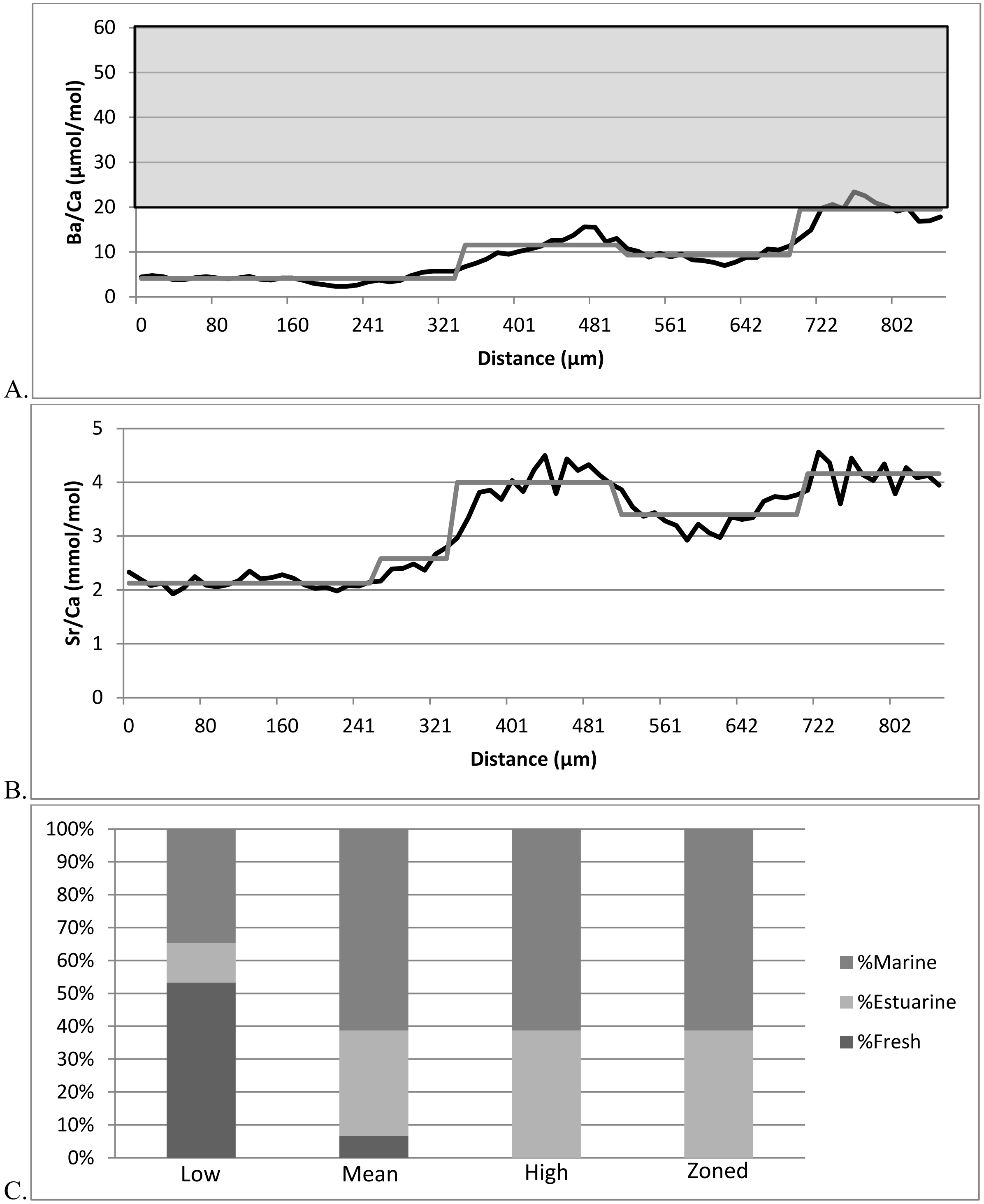
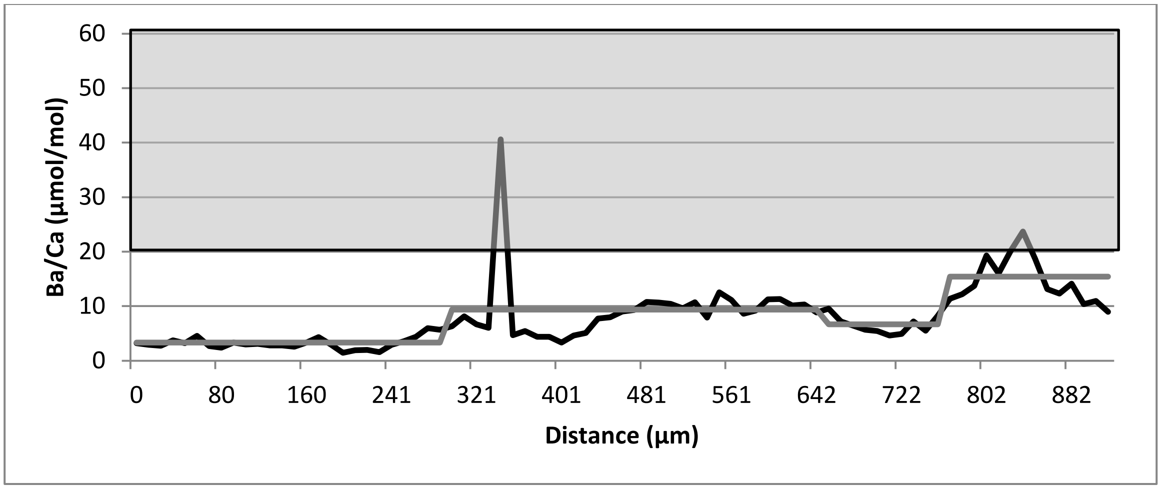
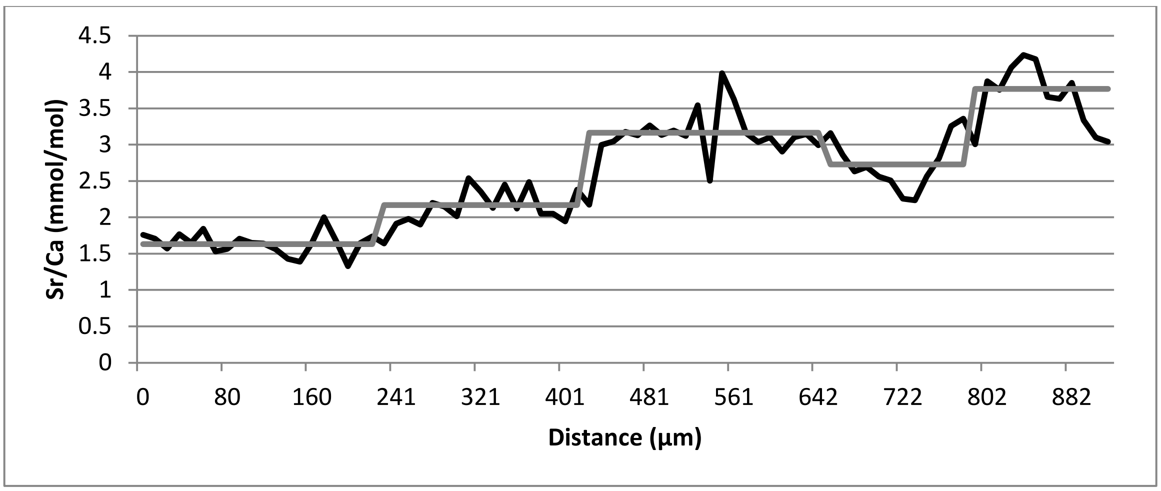


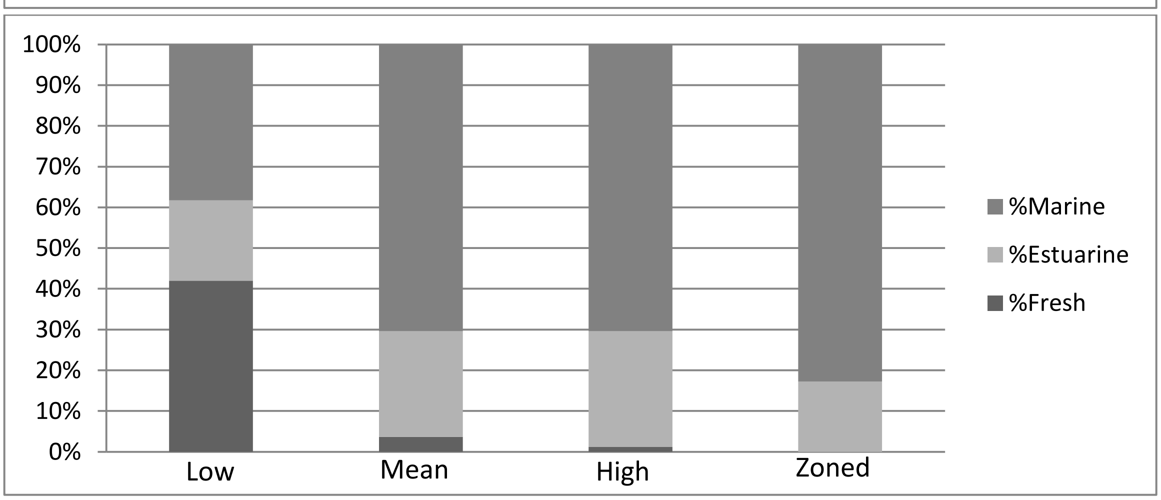
Figure AB.160. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 235. Figure 160.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.161. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 237. Figure 161.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

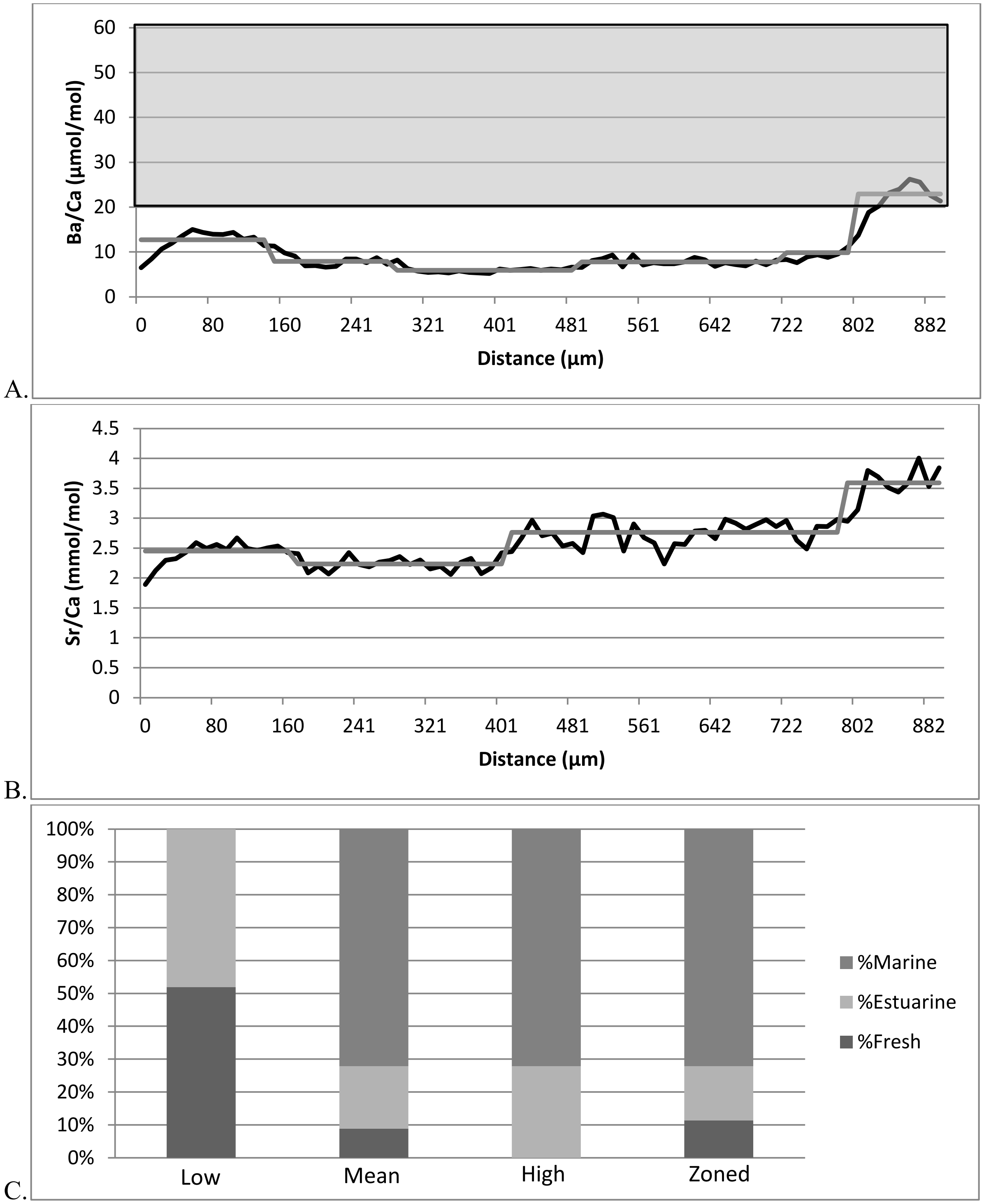
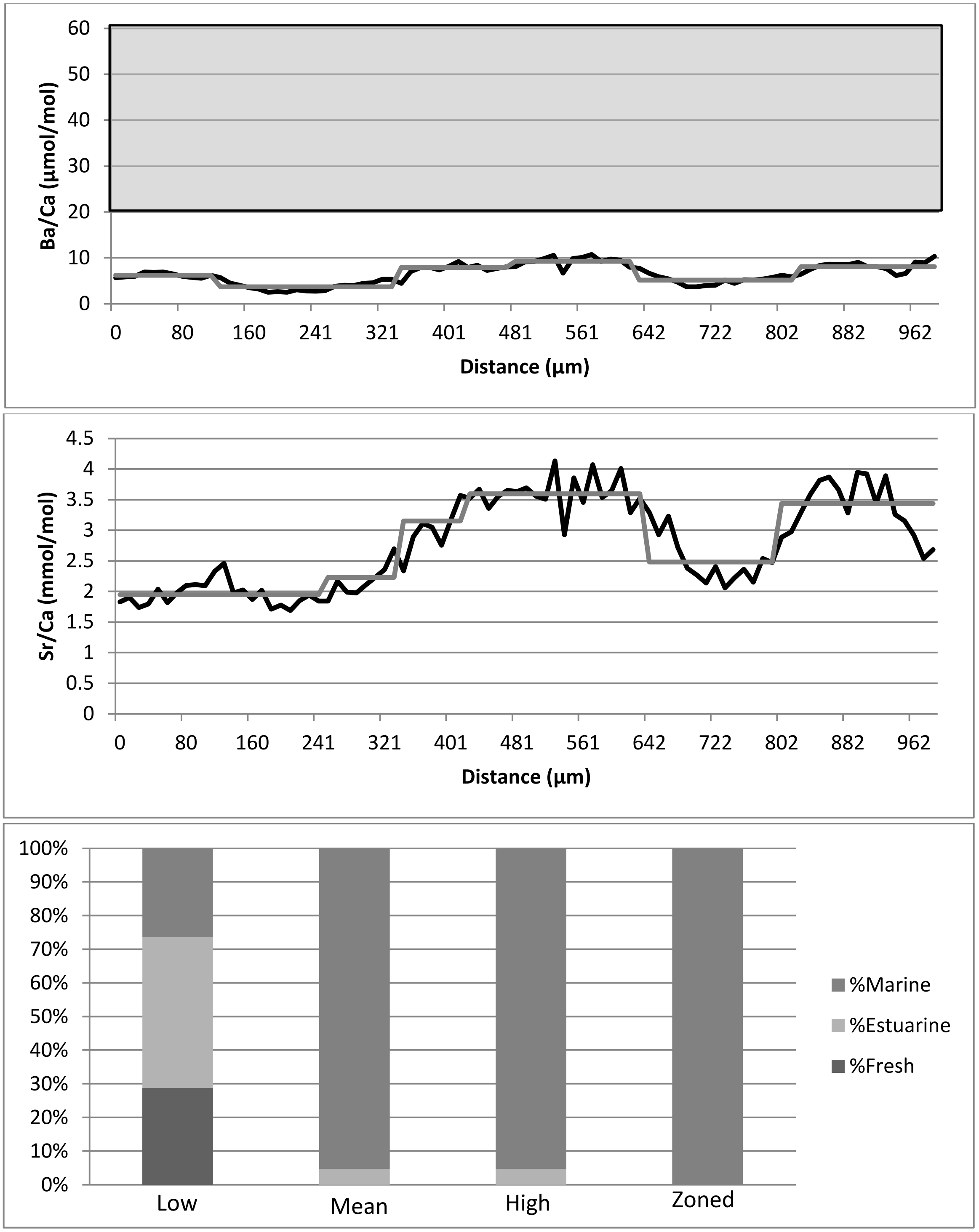


Figure AB.162. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 239. Figure 162.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

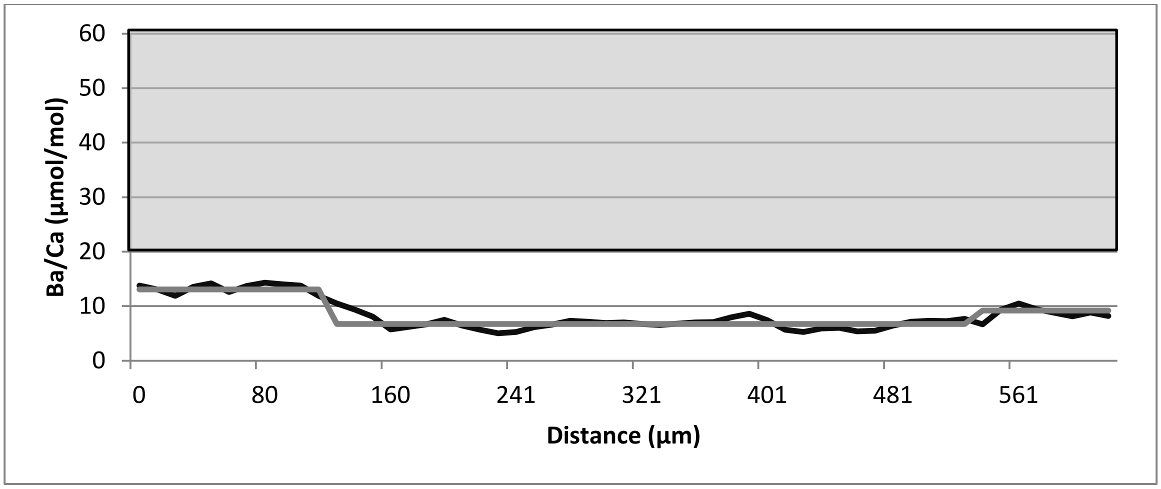


A.

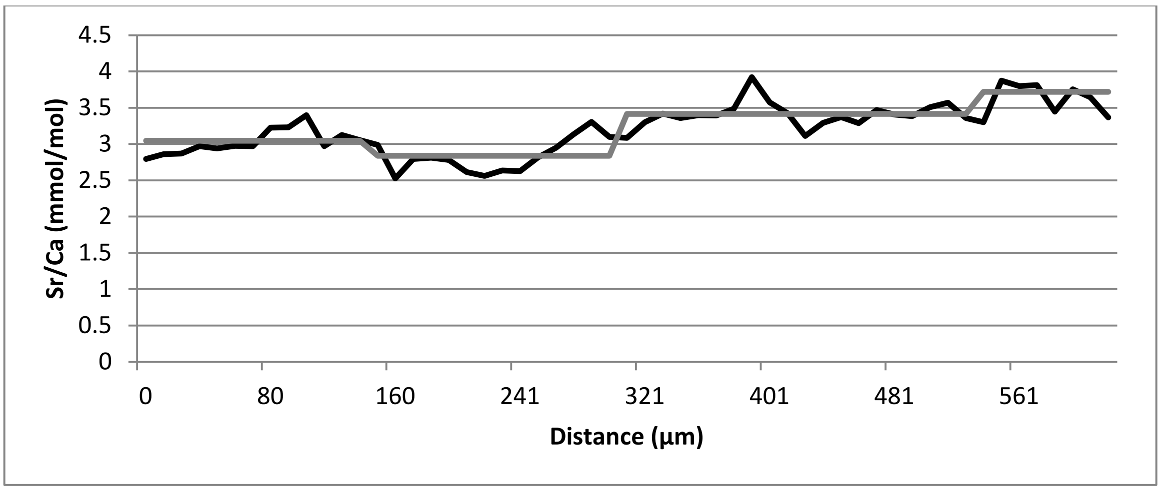
B.

C.

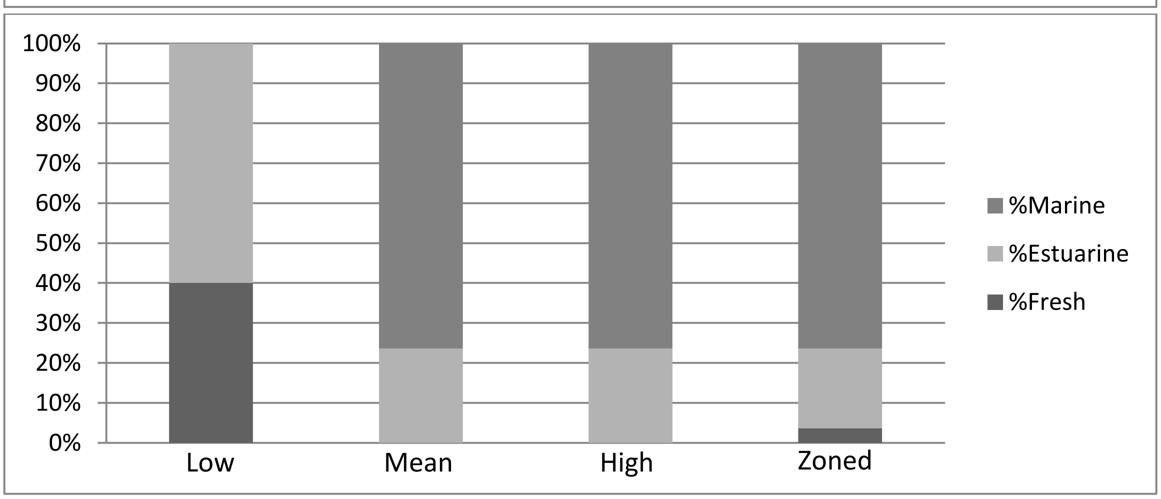
Figure AB.163. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 324. Figure 163.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.164. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 408. Figure 164.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

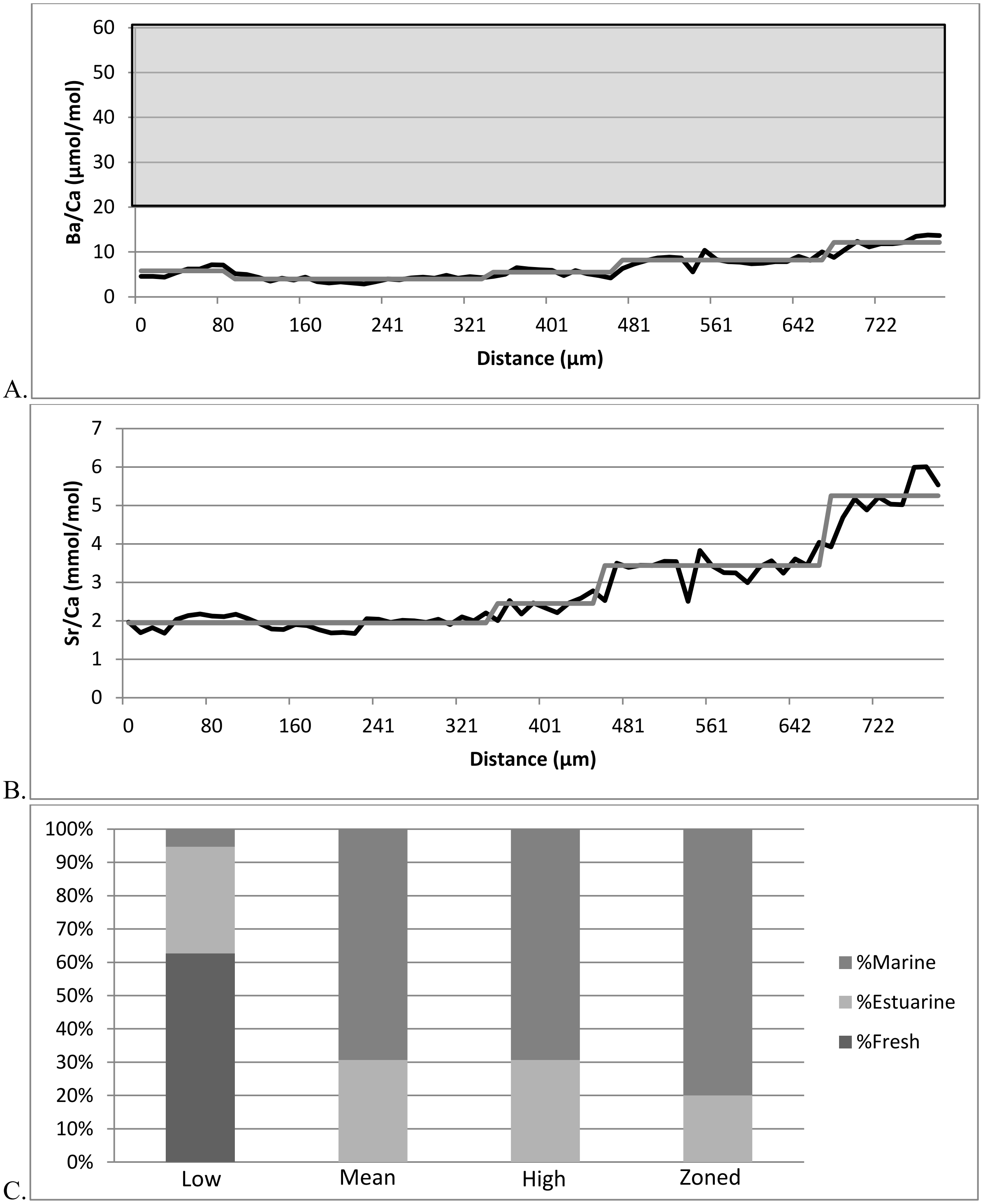
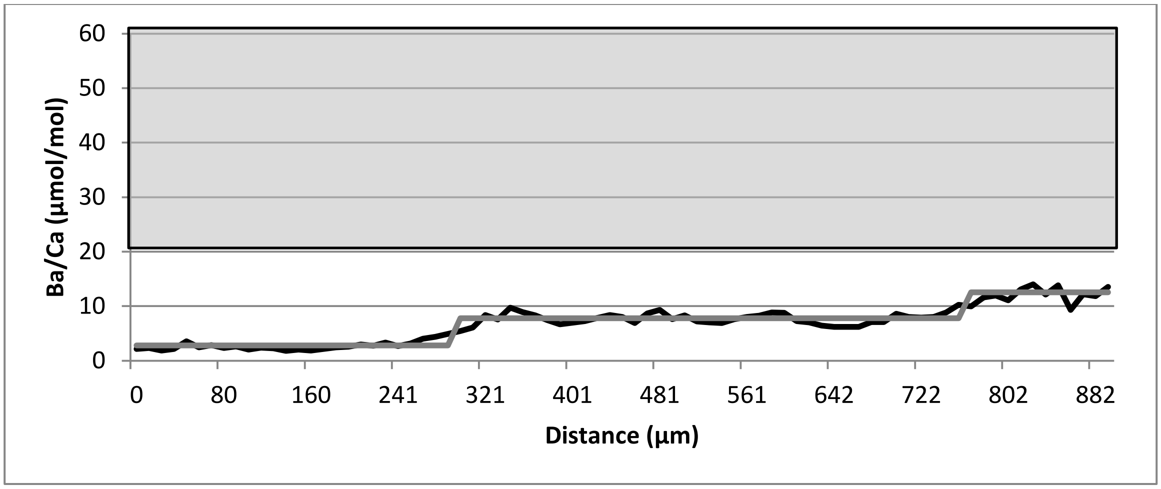
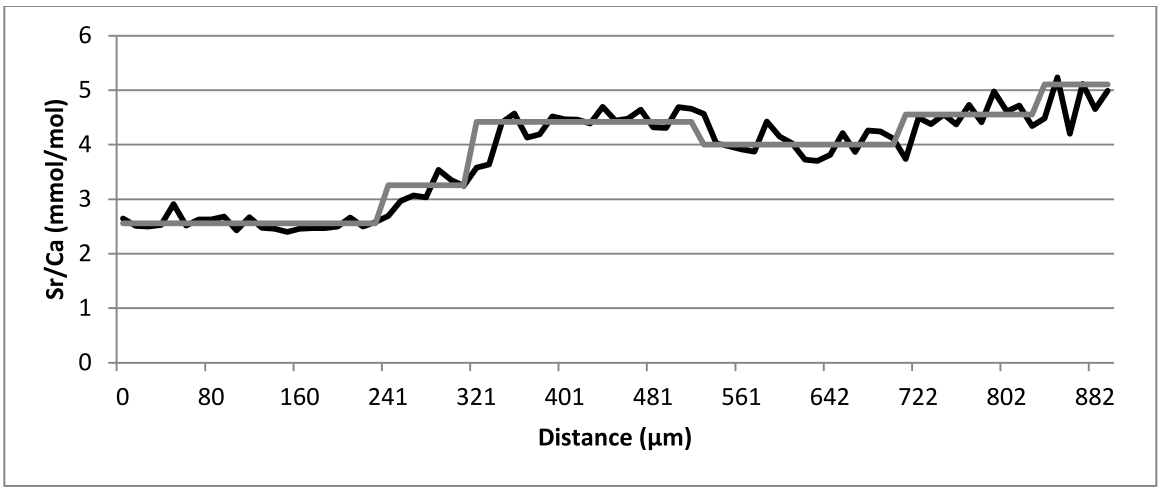


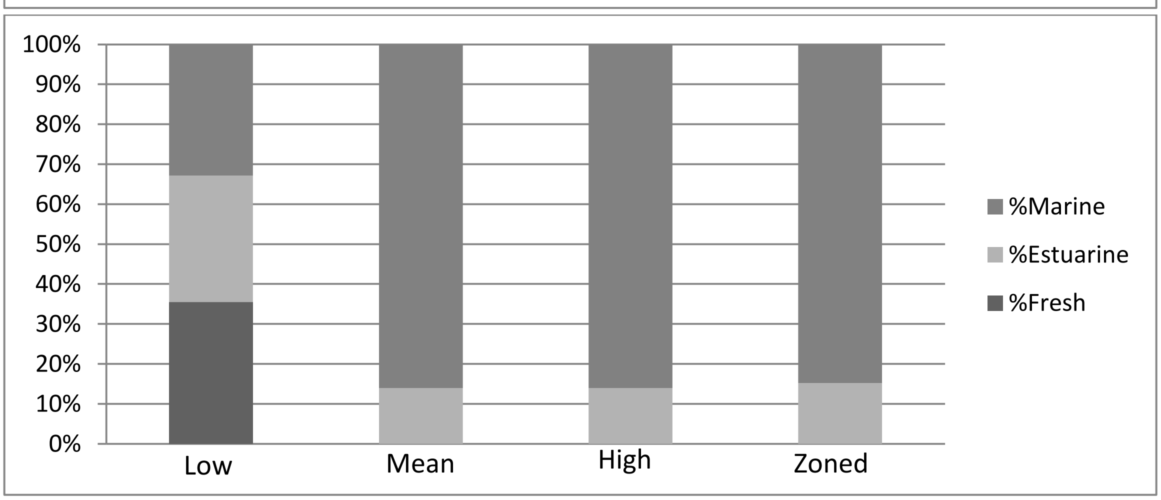
Figure AB.165. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 409. Figure 165.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

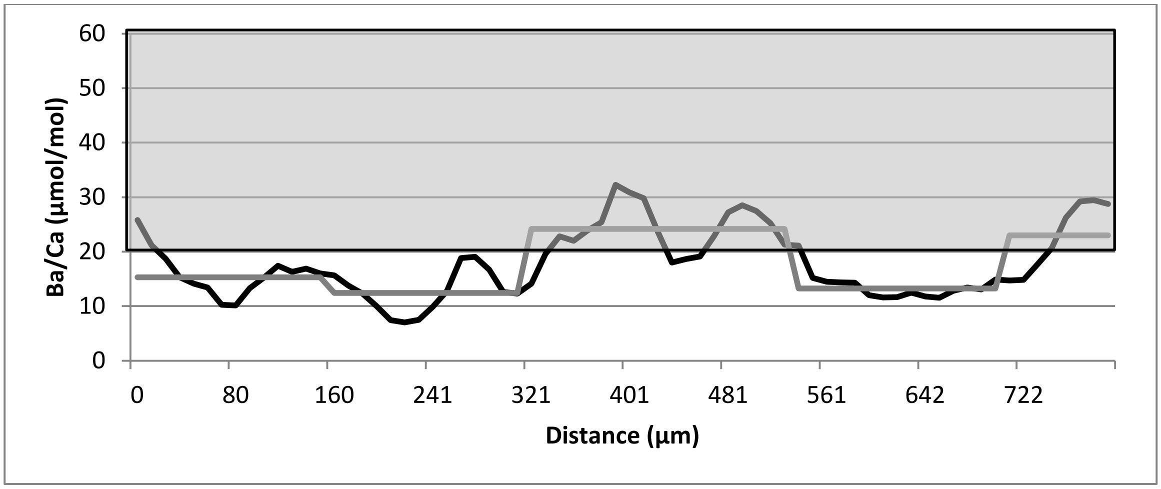


B.

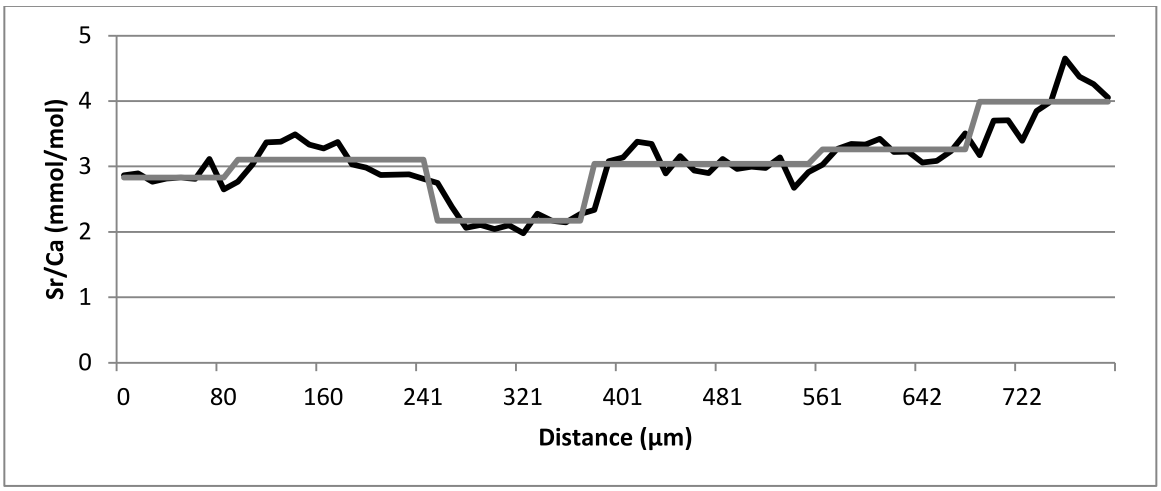


C.

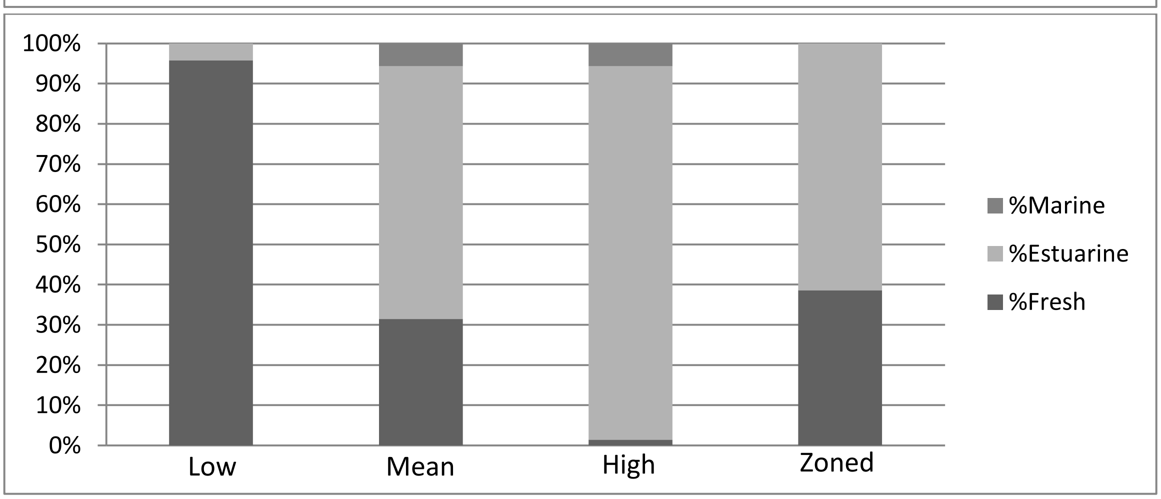
Figure AB.166. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 410. Figure 166.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

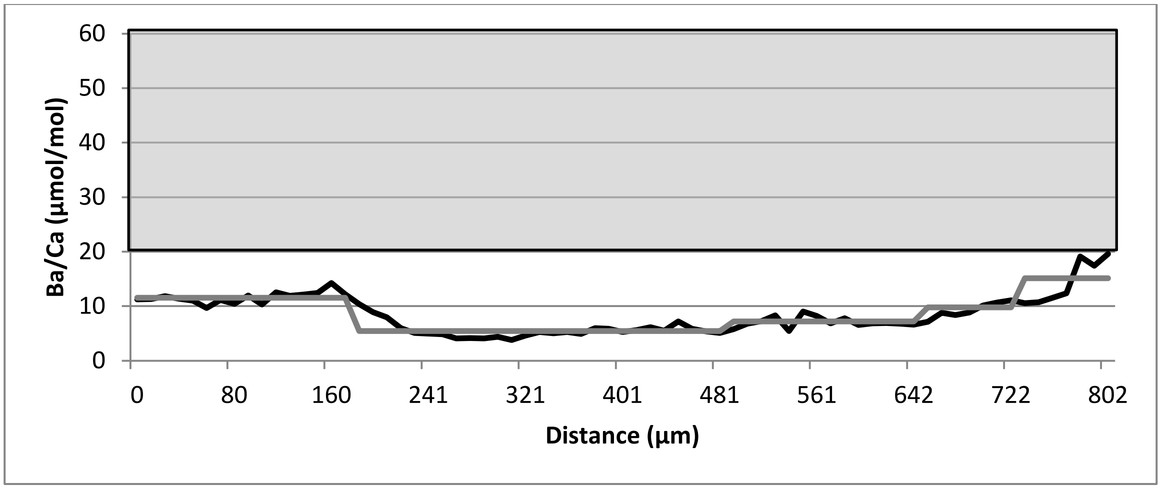


B.

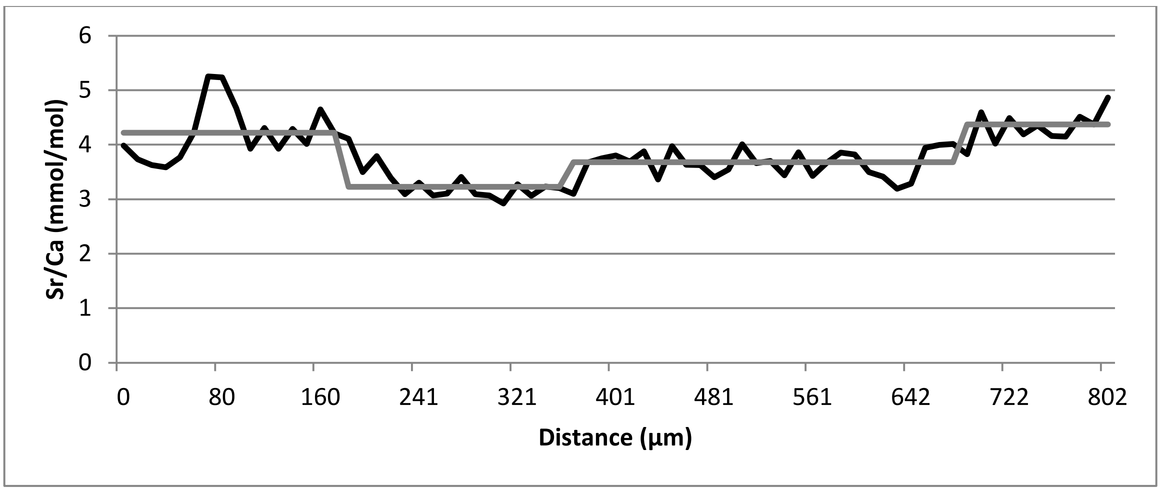


C.

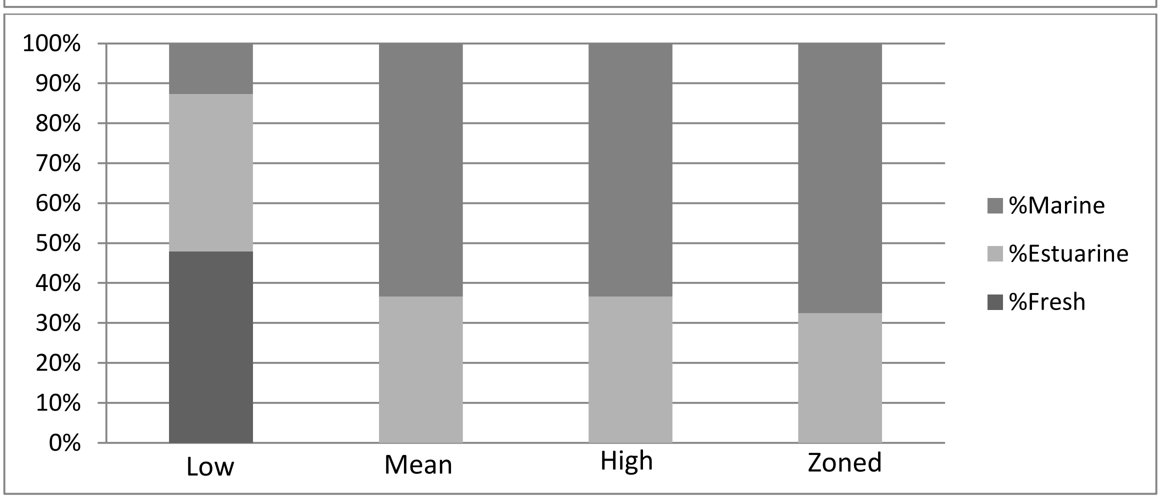
Figure AB.167. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 412. Figure 167.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

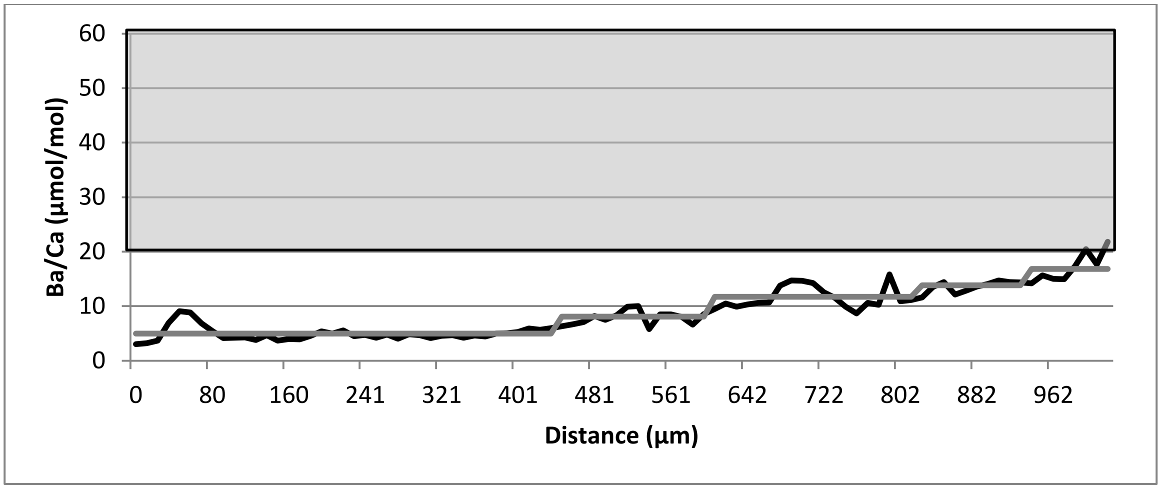


B.

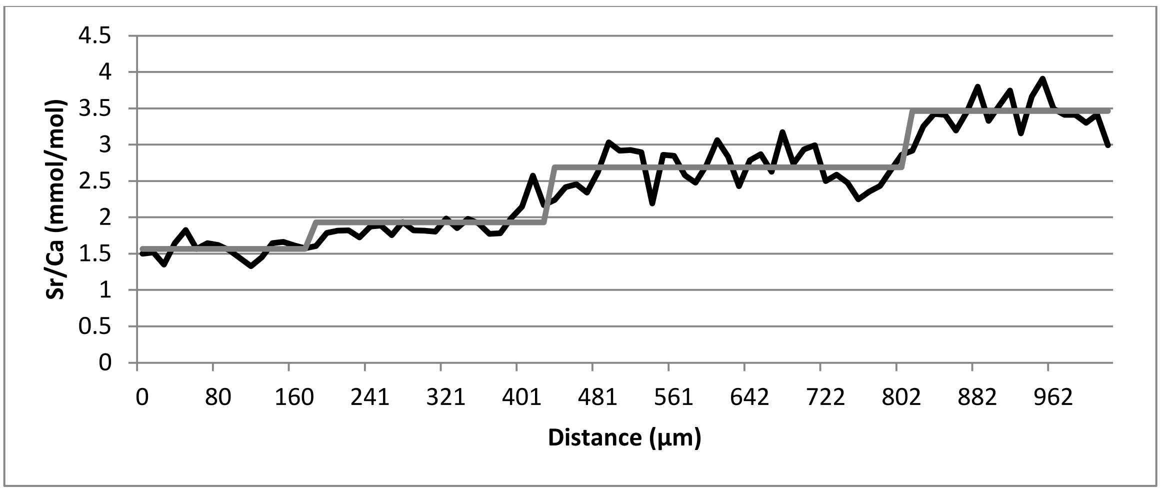


C.

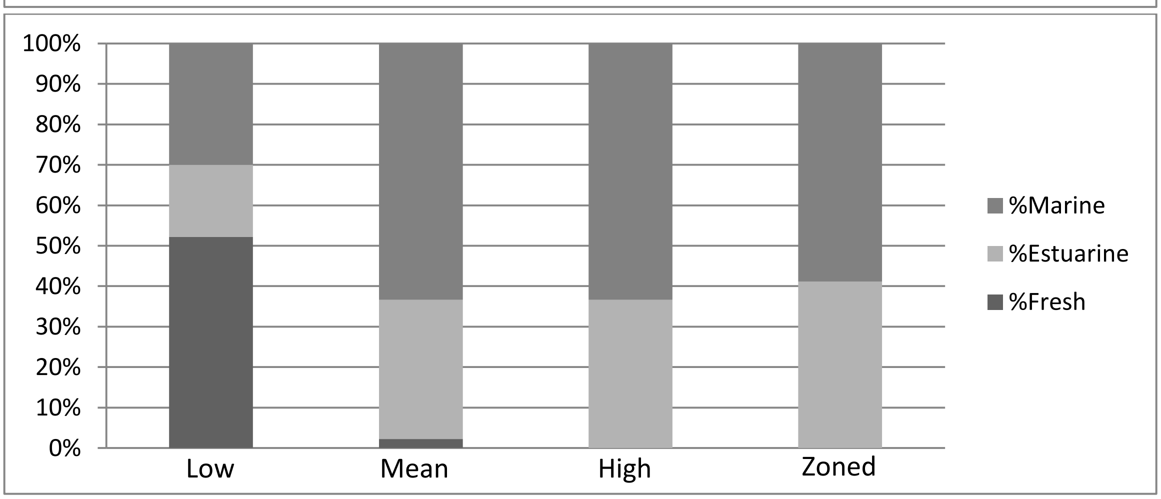
Figure AB.168. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 416. Figure 168.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.169. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 417. Figure 169.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

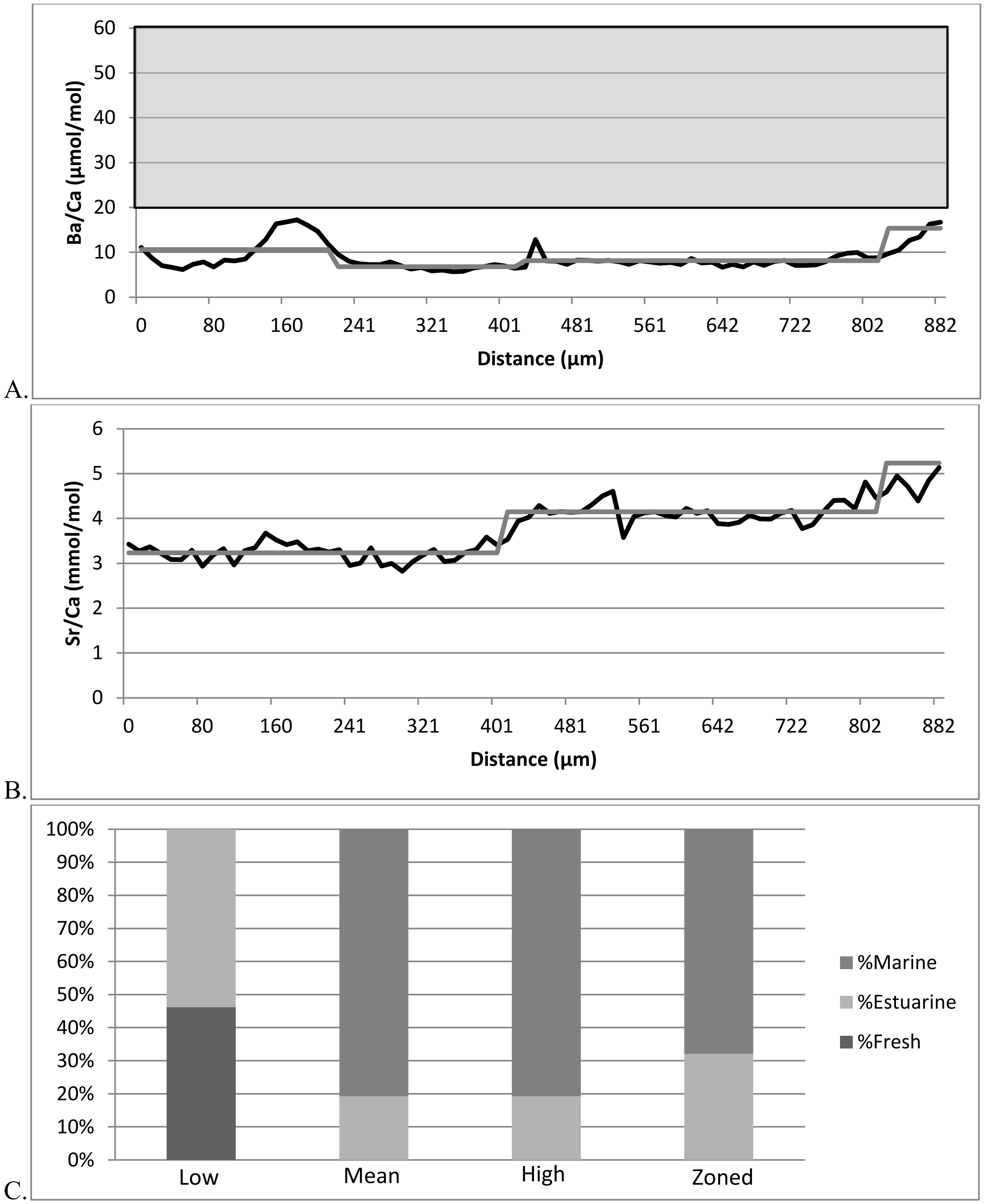
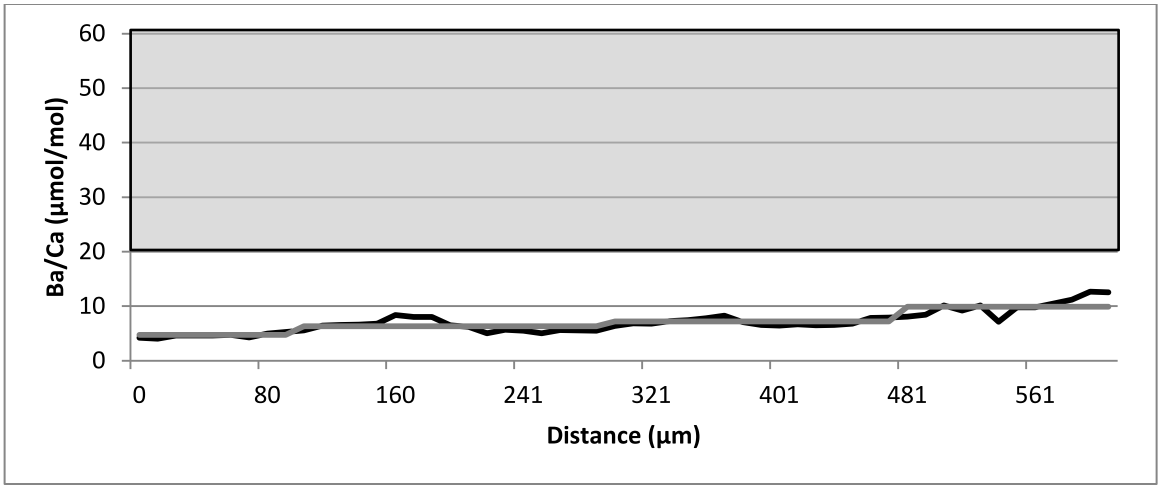
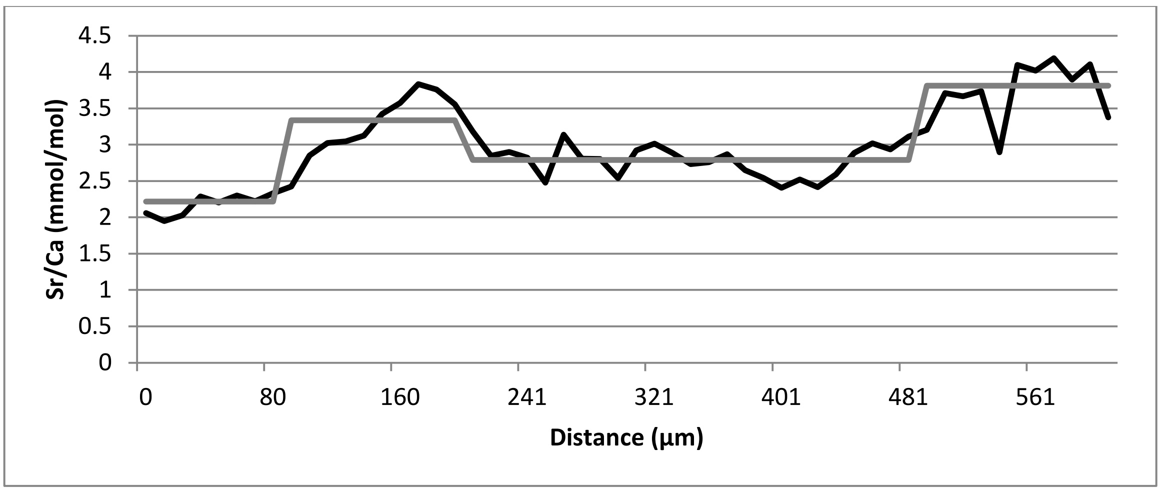


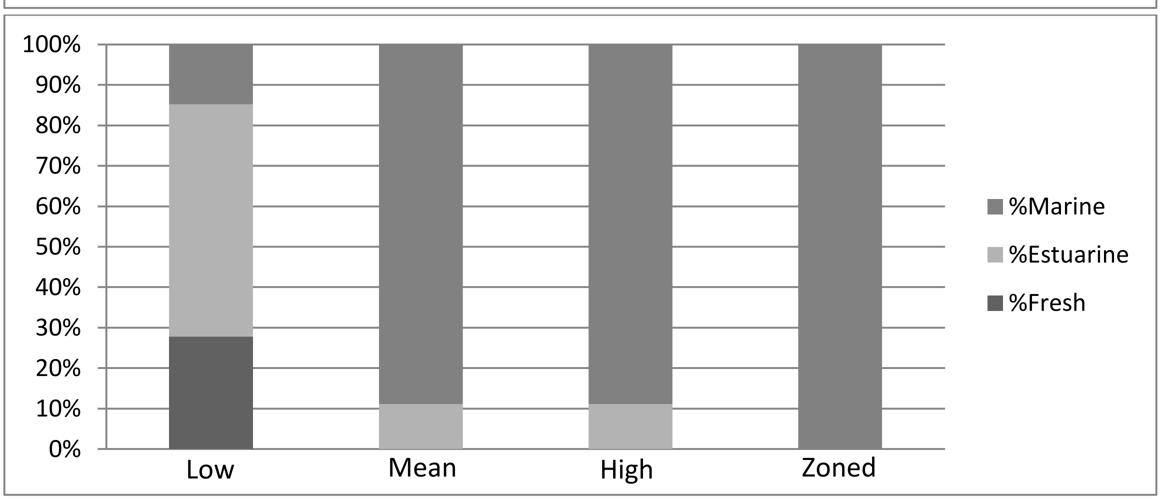
Figure AB.170. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 420. Figure 170.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.171. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 421. Figure 171.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

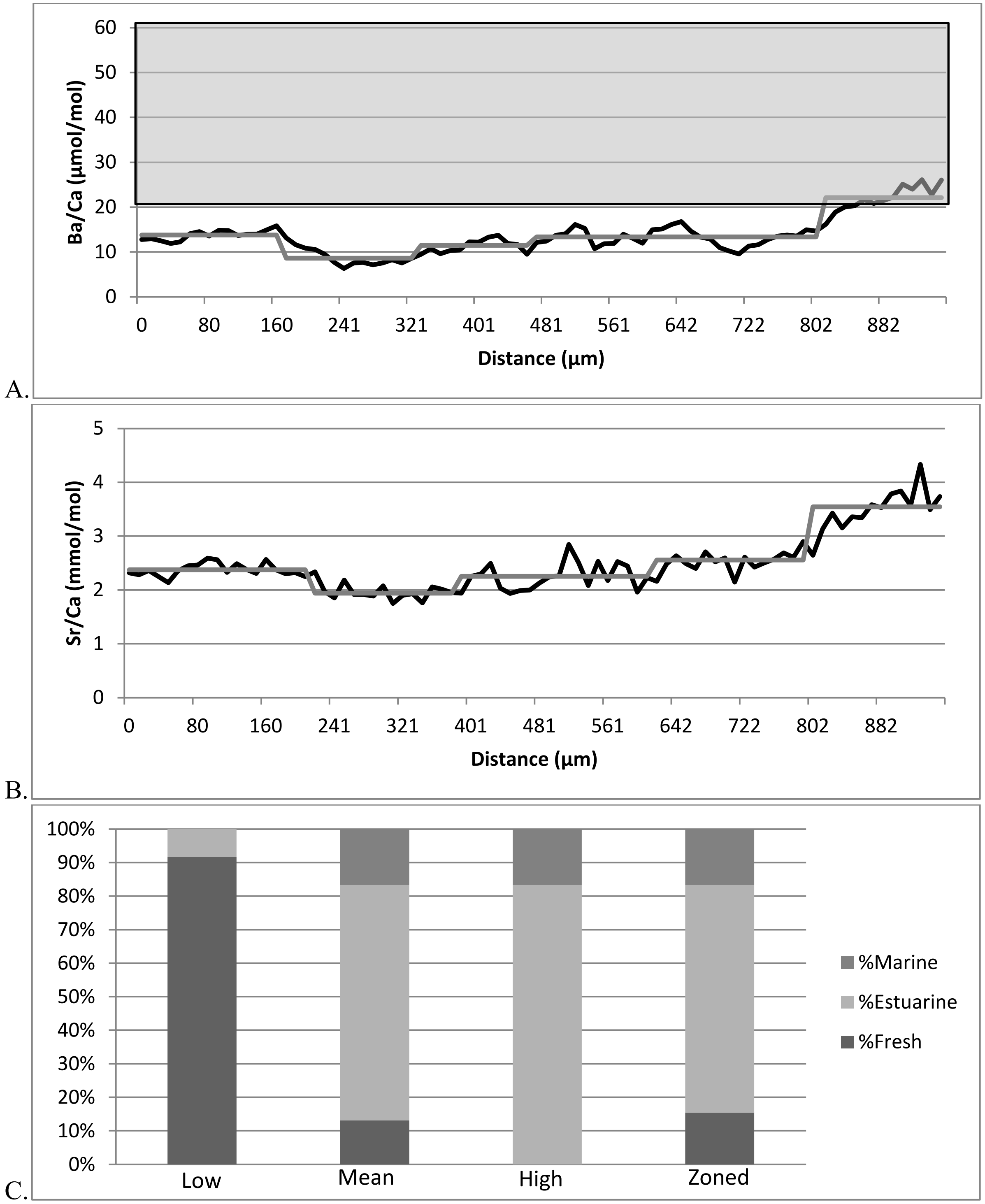


Figure AB.172. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 421. Figure 172.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

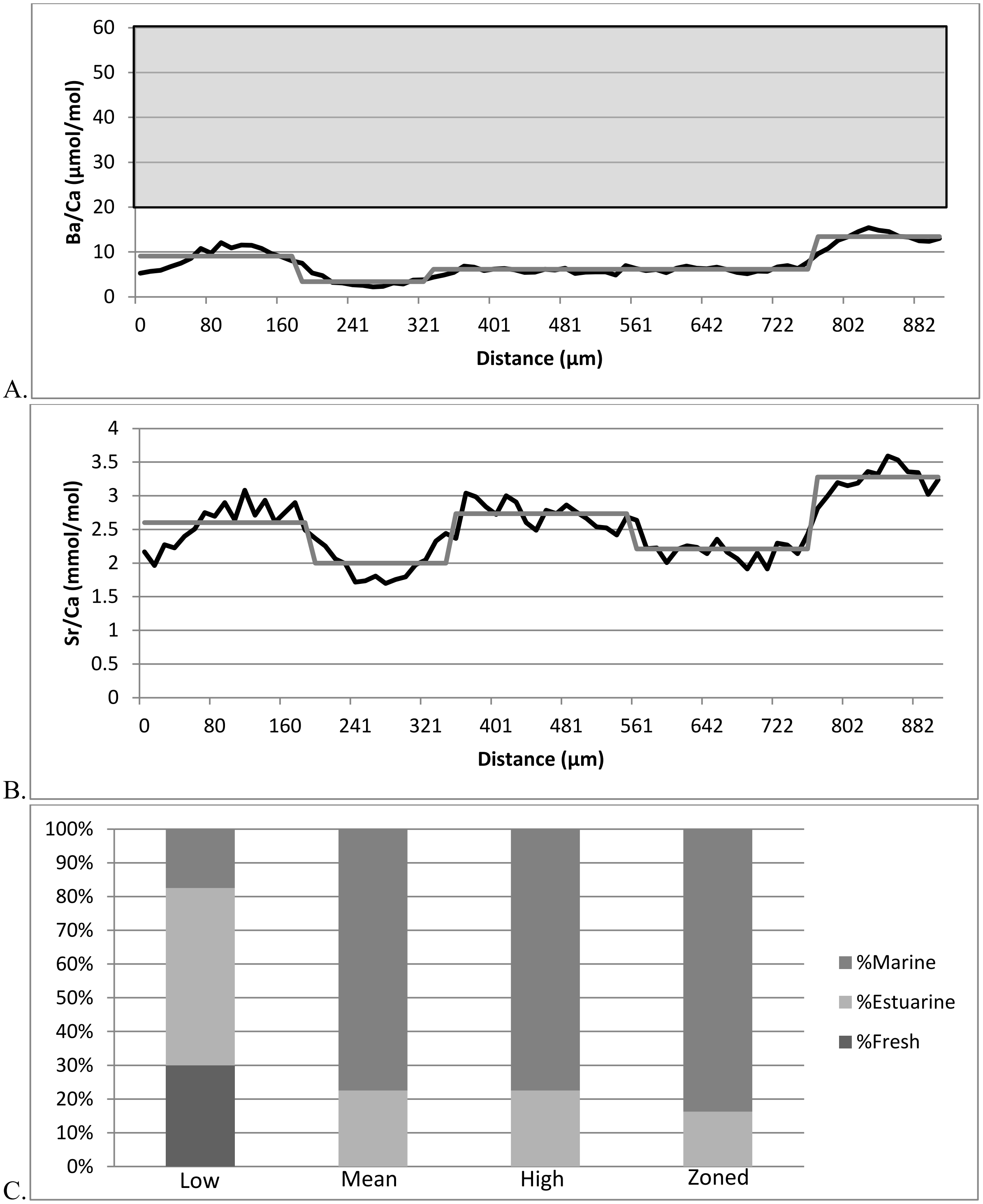
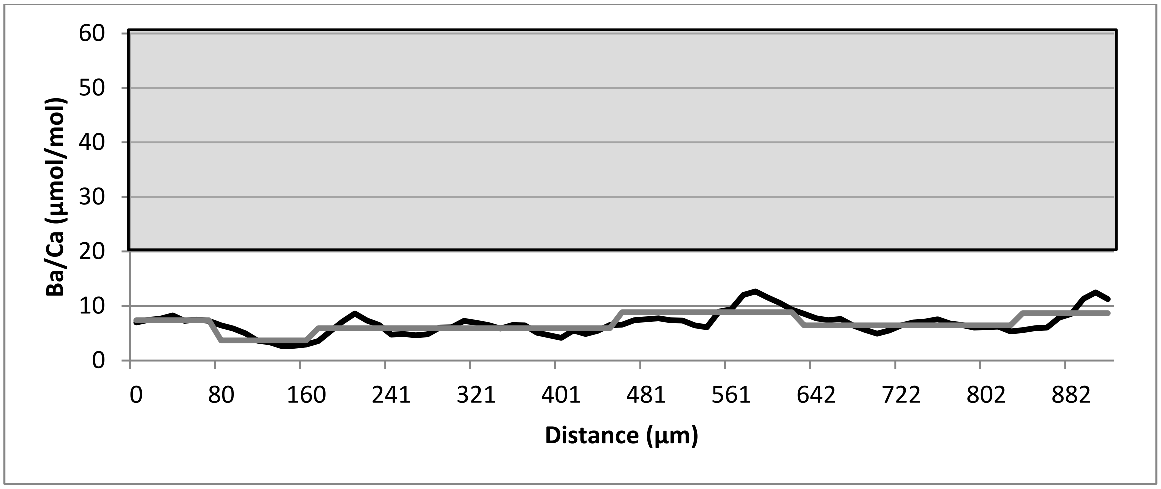
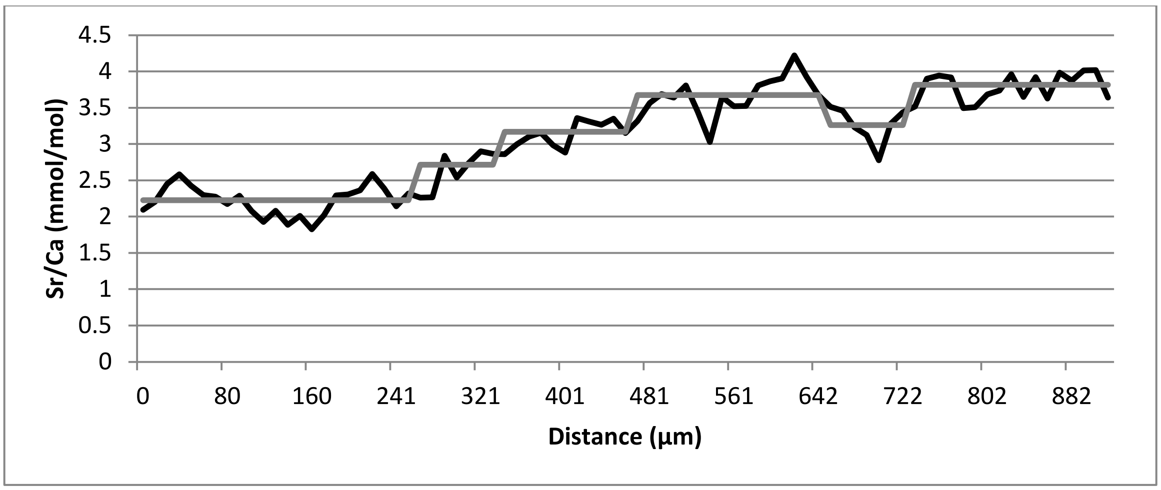


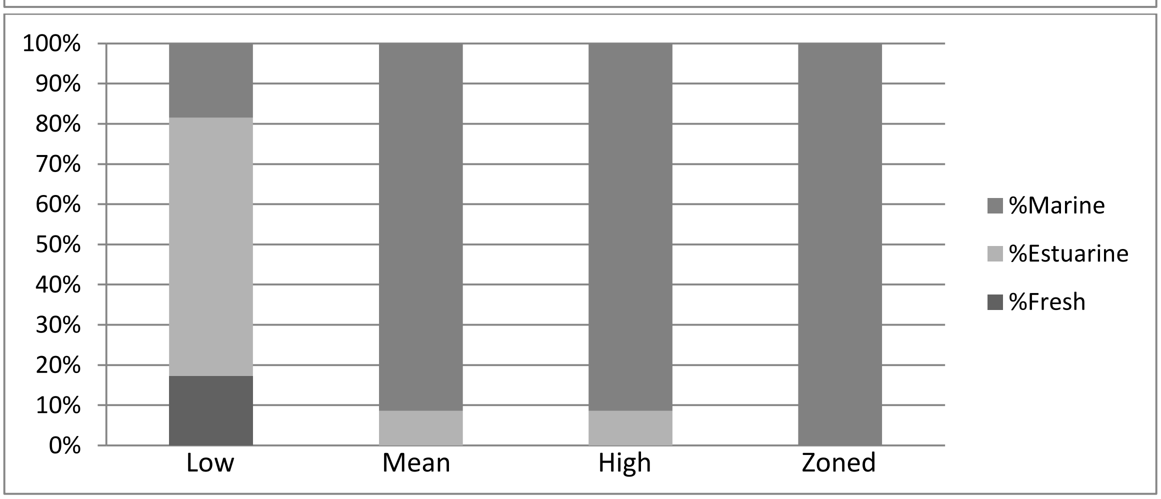
Figure AB.173. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 423. Figure 173.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.174. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 425. Figure 174.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

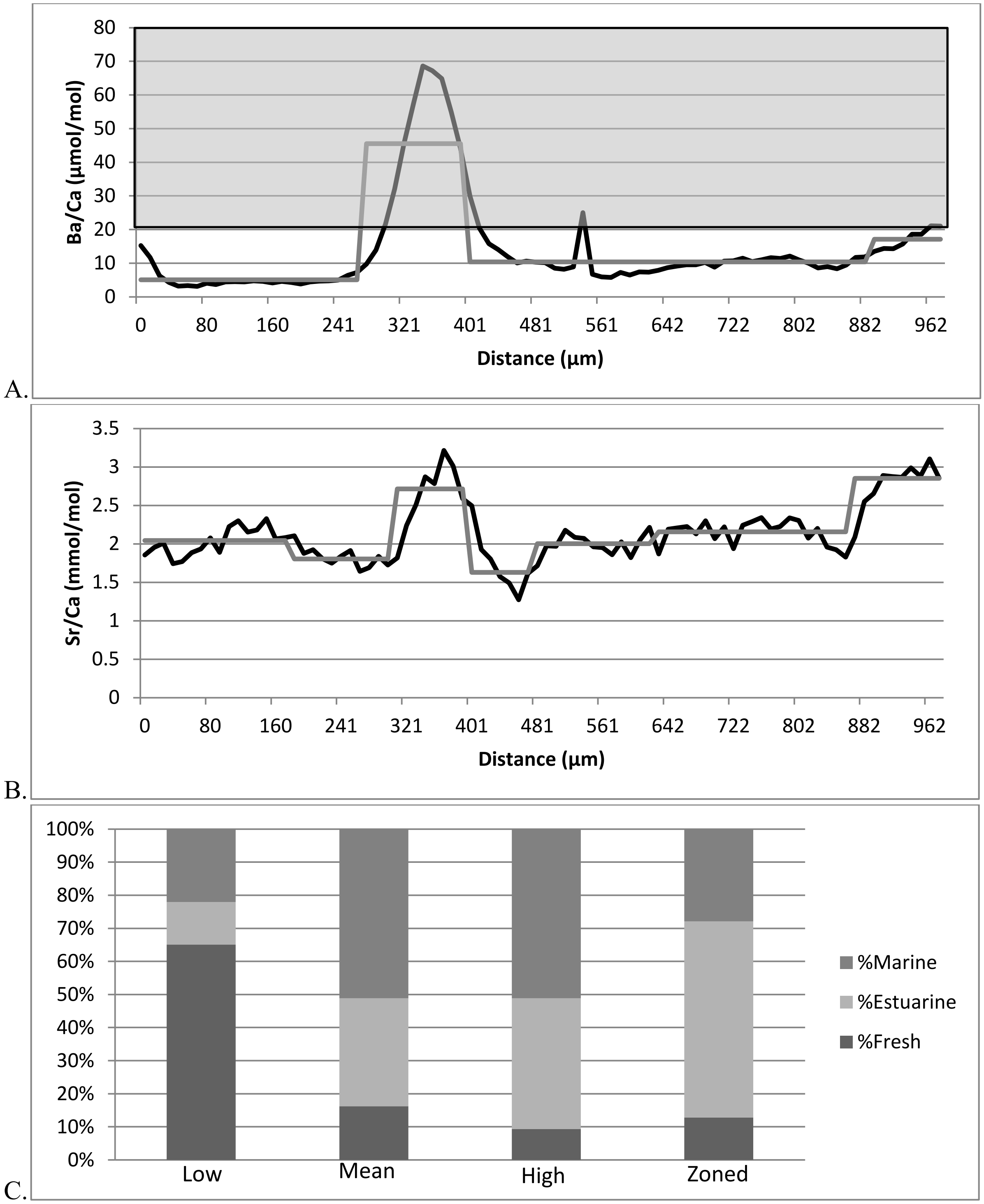
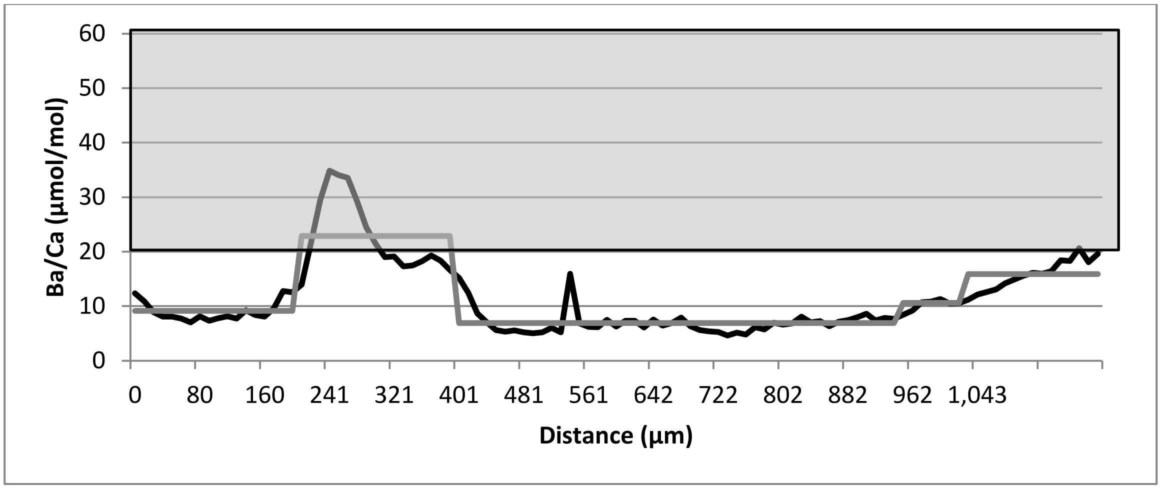
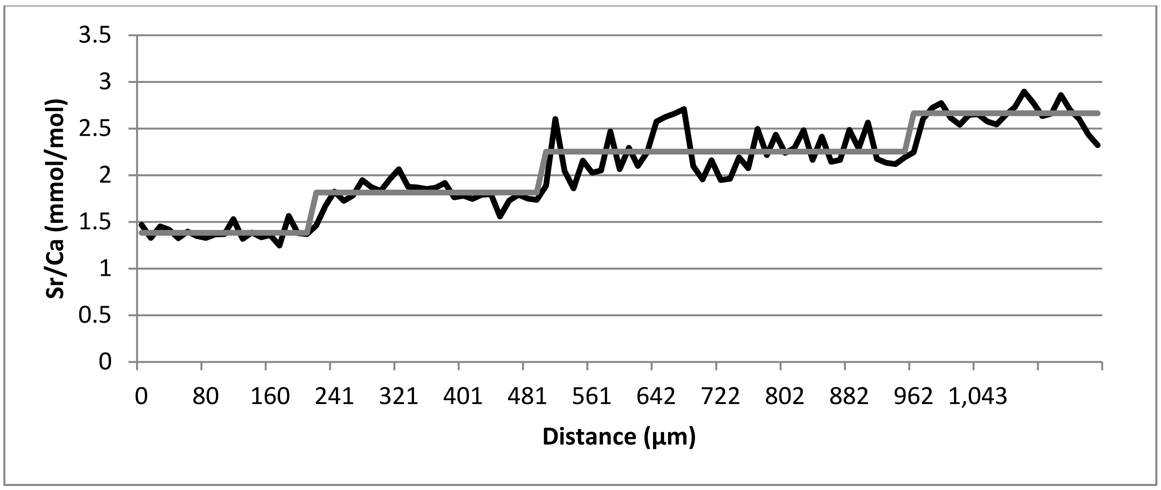


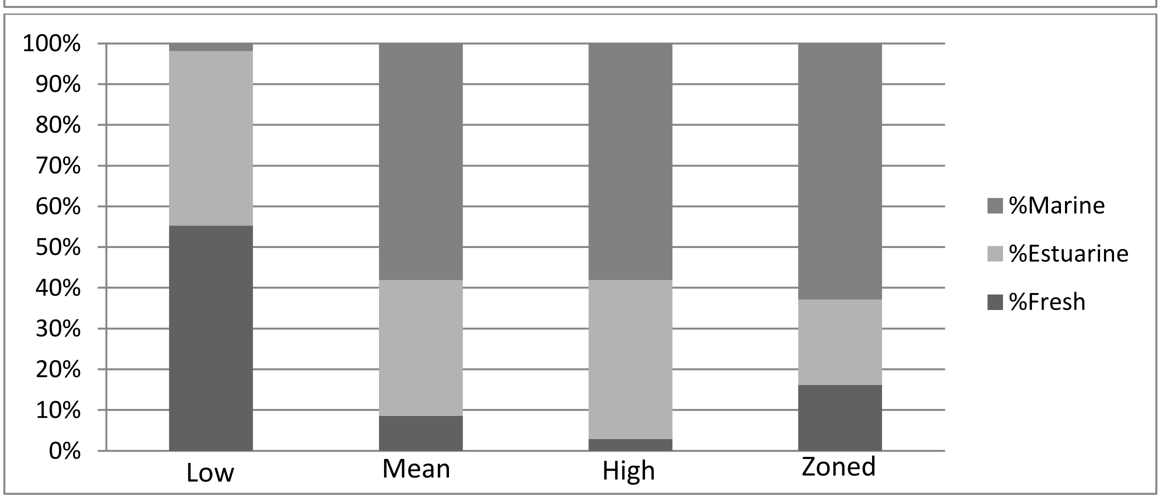
Figure AB.175. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 428. Figure 175.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

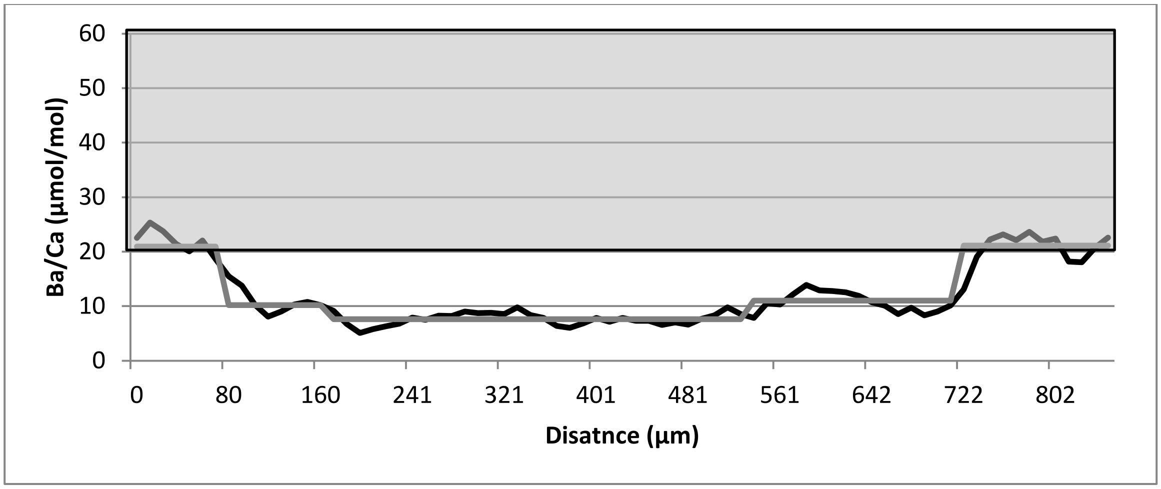


B.

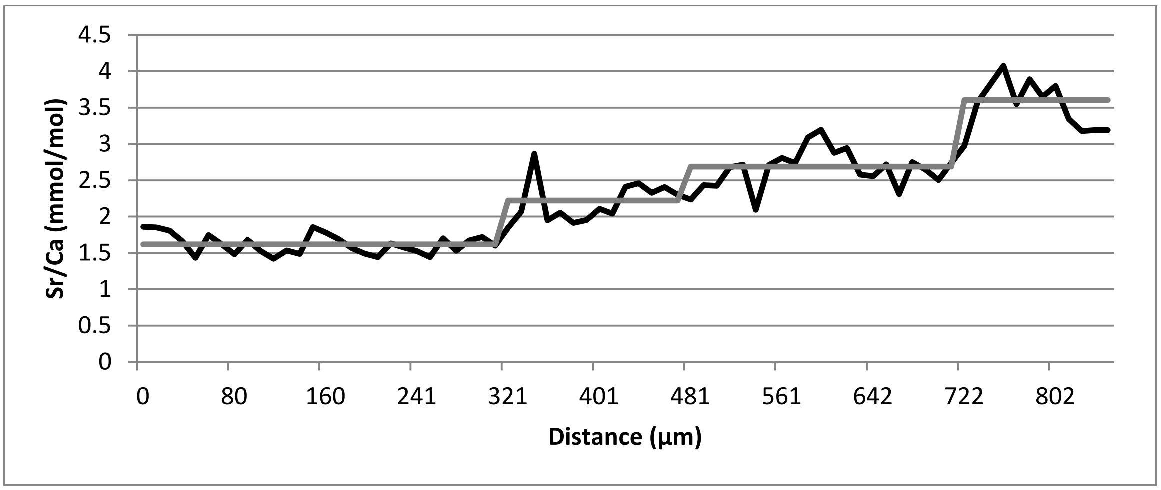


C.

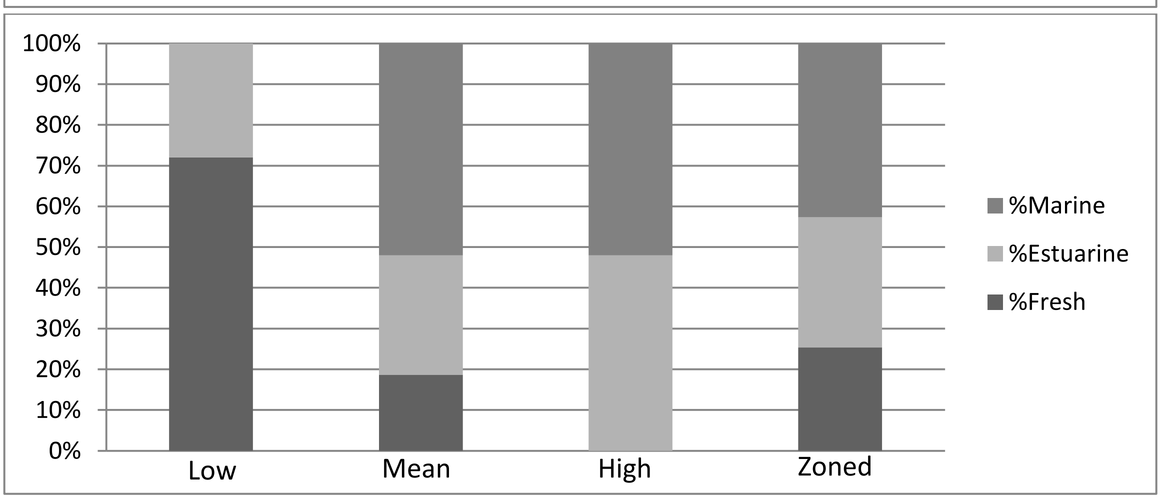
Figure AB.176. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 429. Figure 176.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

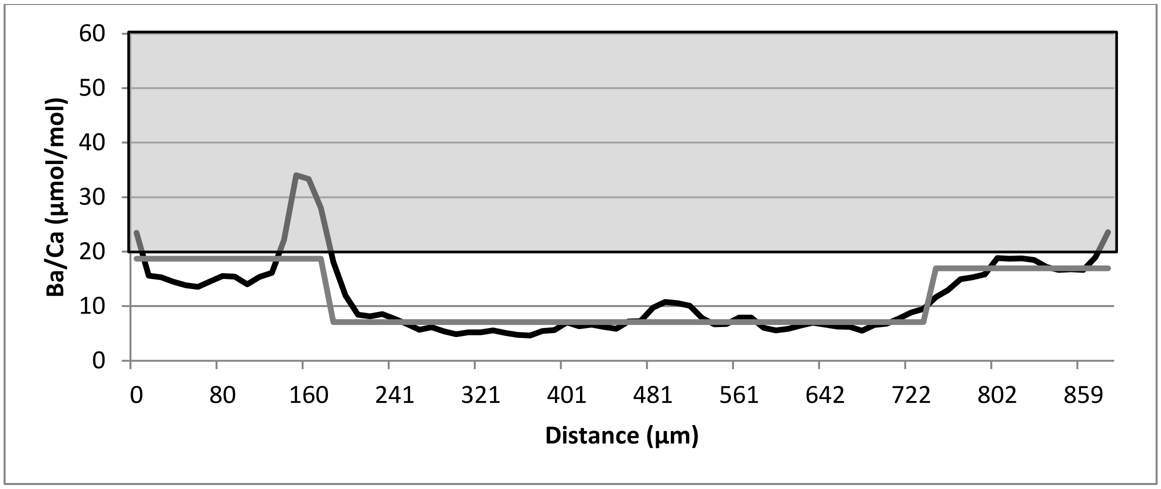


B.

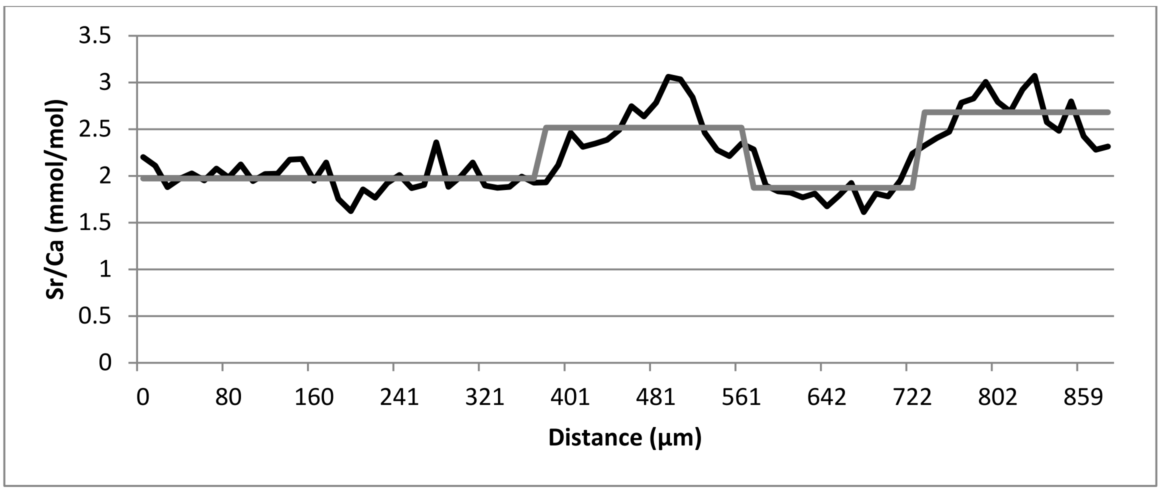


C.

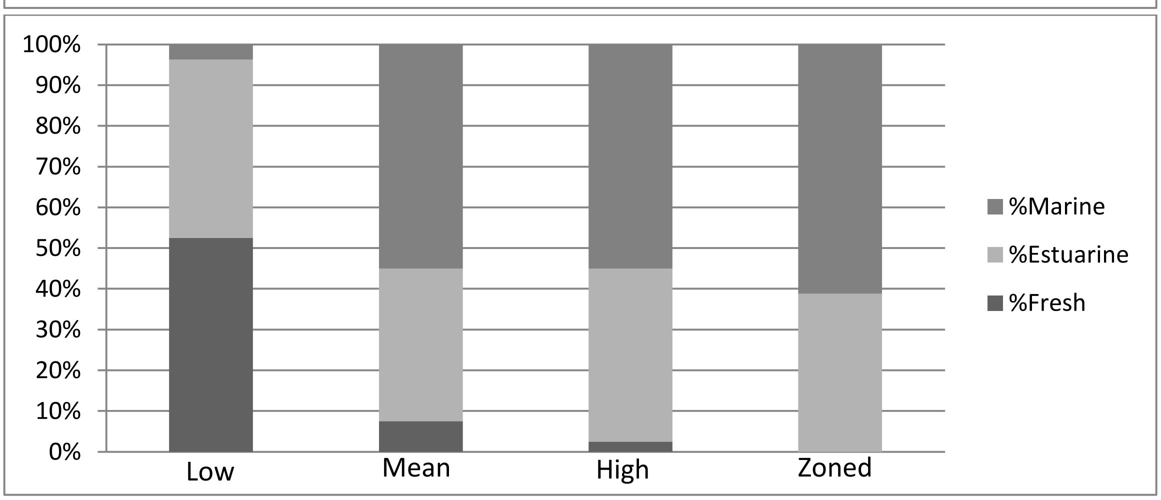
Figure AB.177. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 430. Figure 177.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

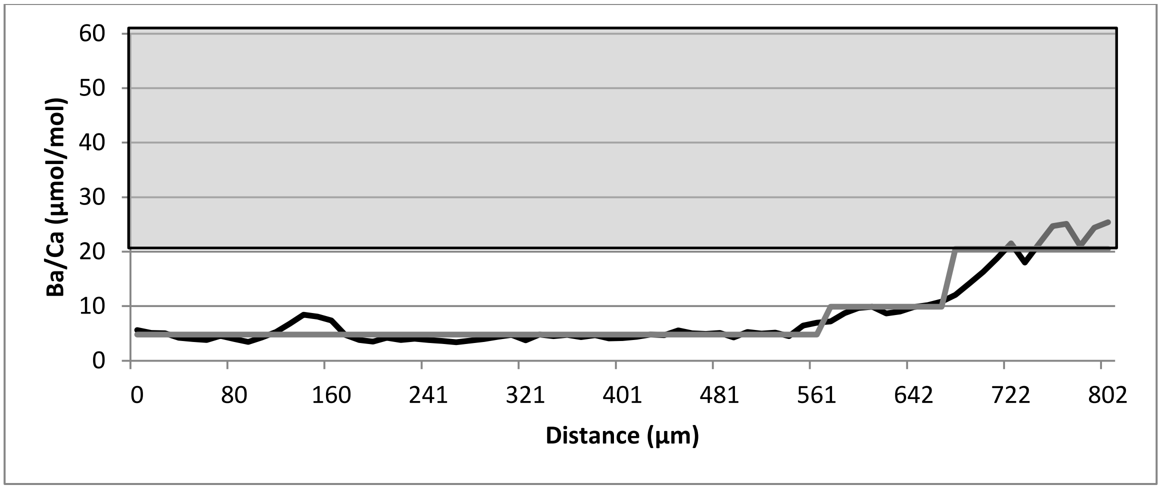


B.

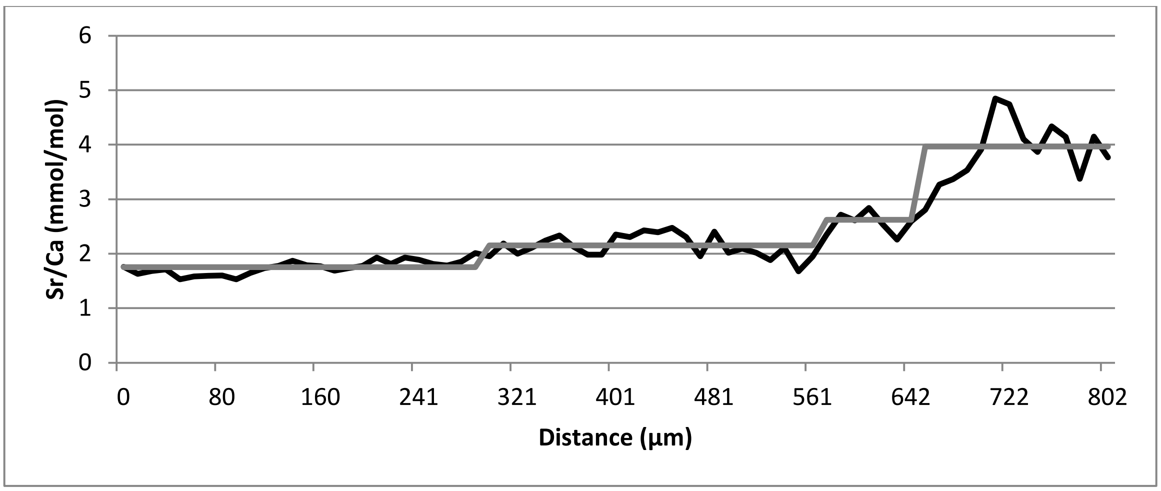


C.

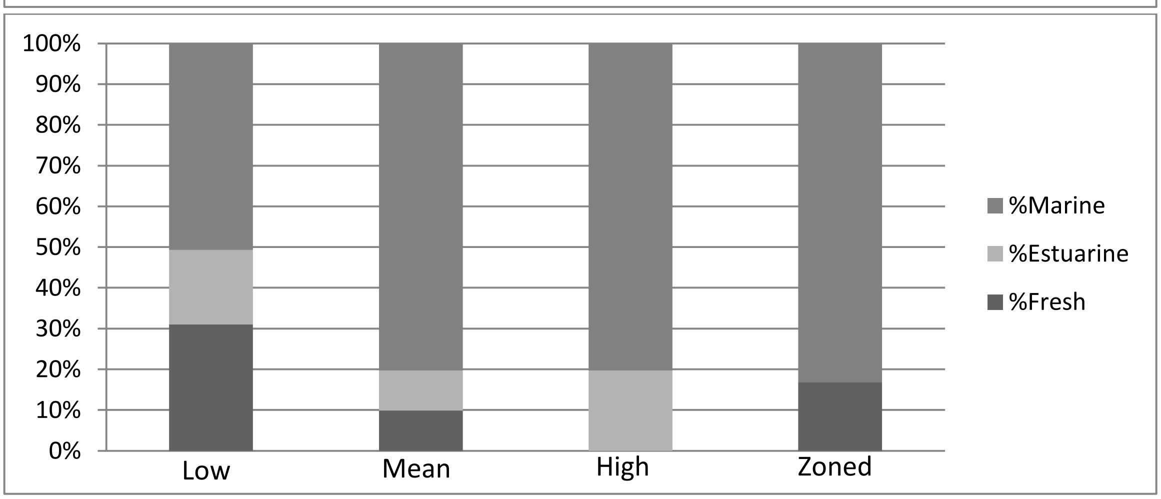
Figure AB.178. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 431. Figure 178.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.179. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 433. Figure 179.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.
B.
C.

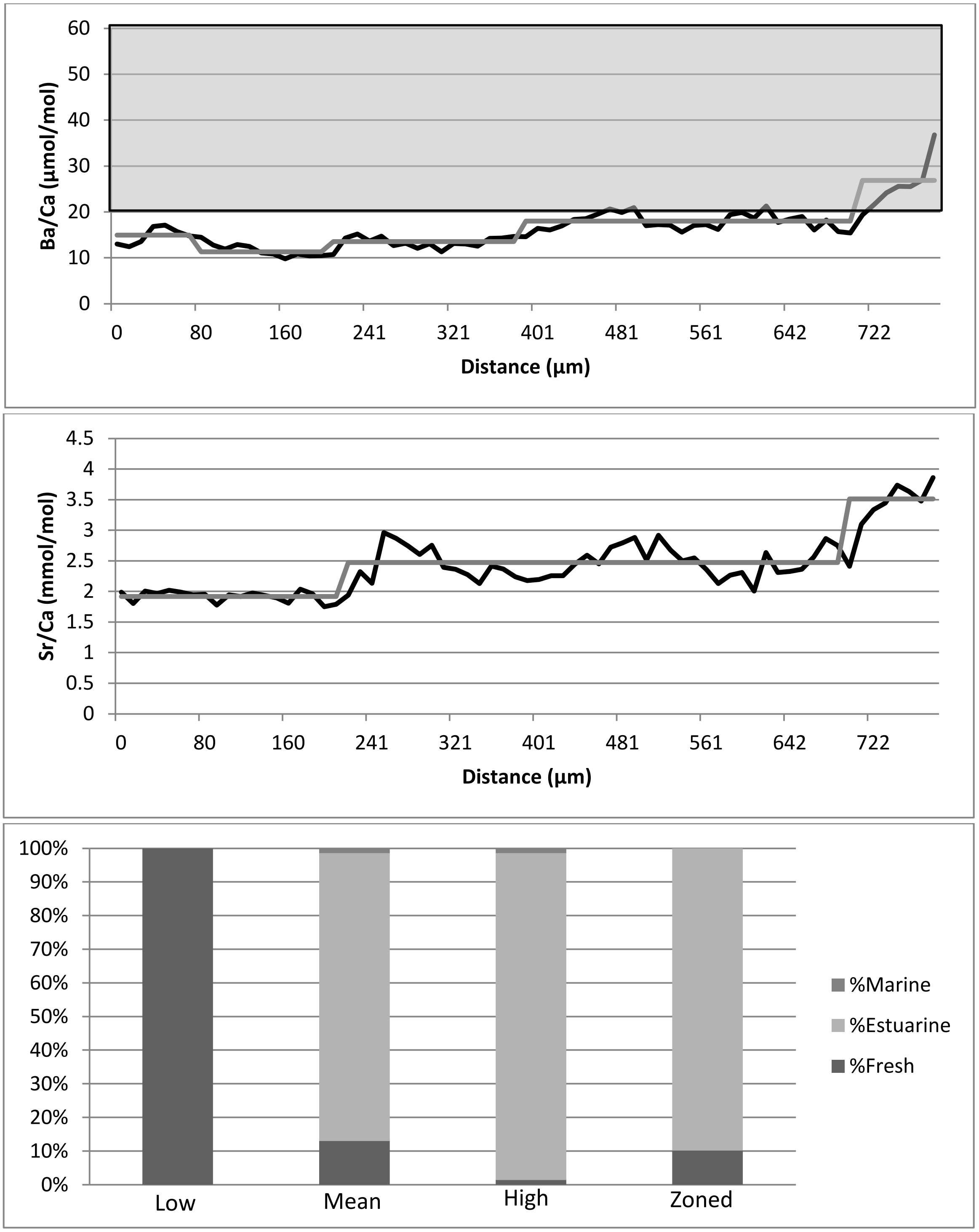
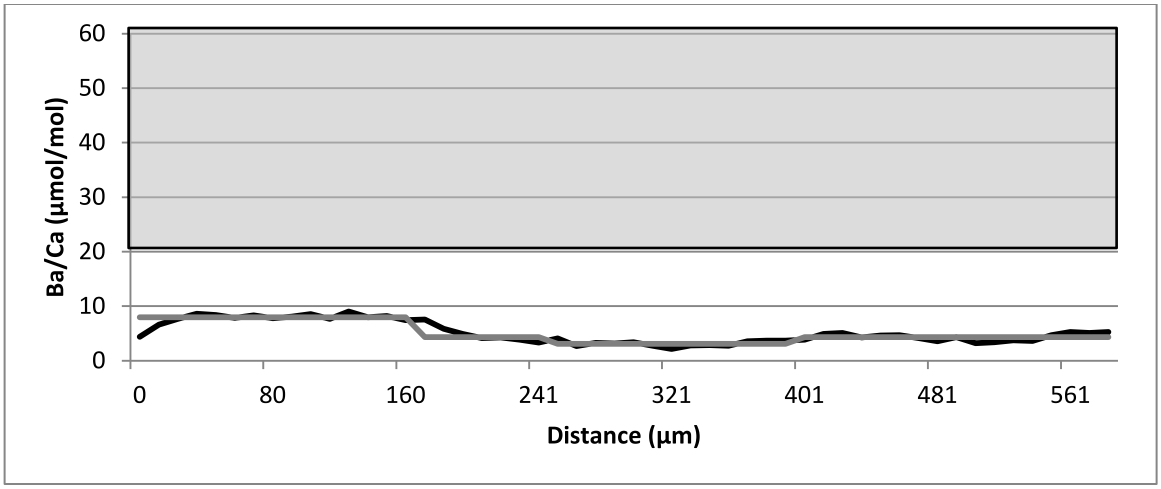
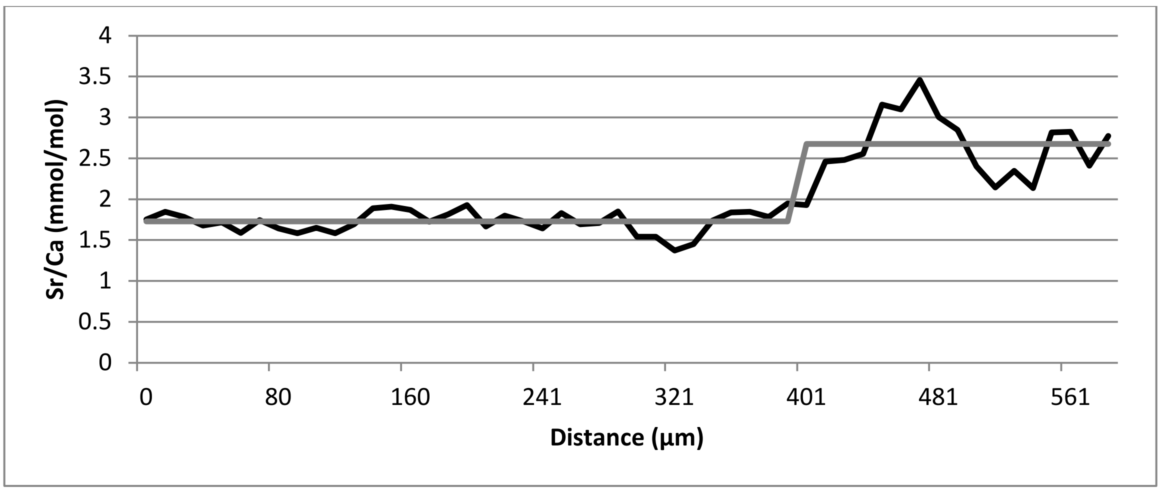


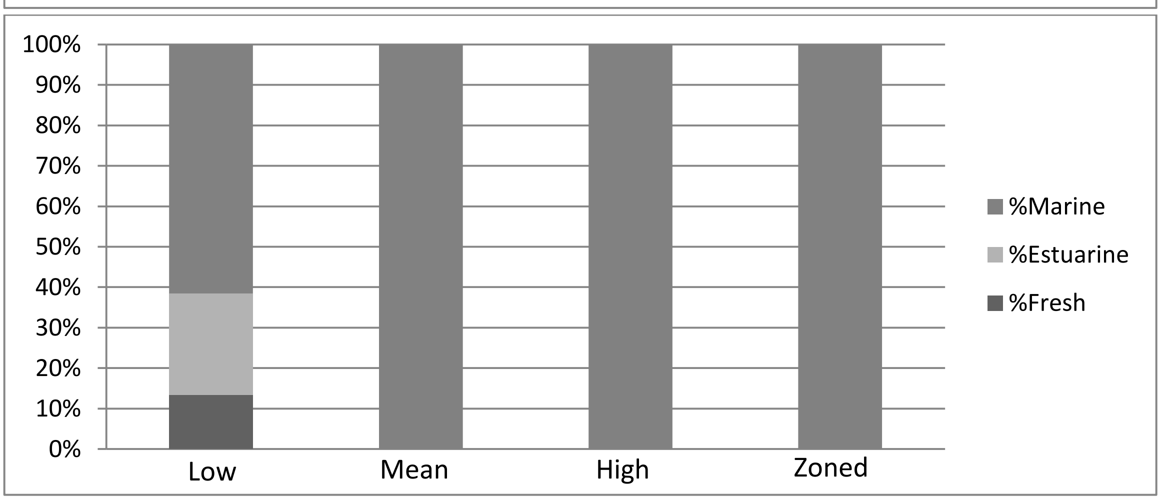
Figure AB.180. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 435. Figure 180.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.181. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 436. Figure 181.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

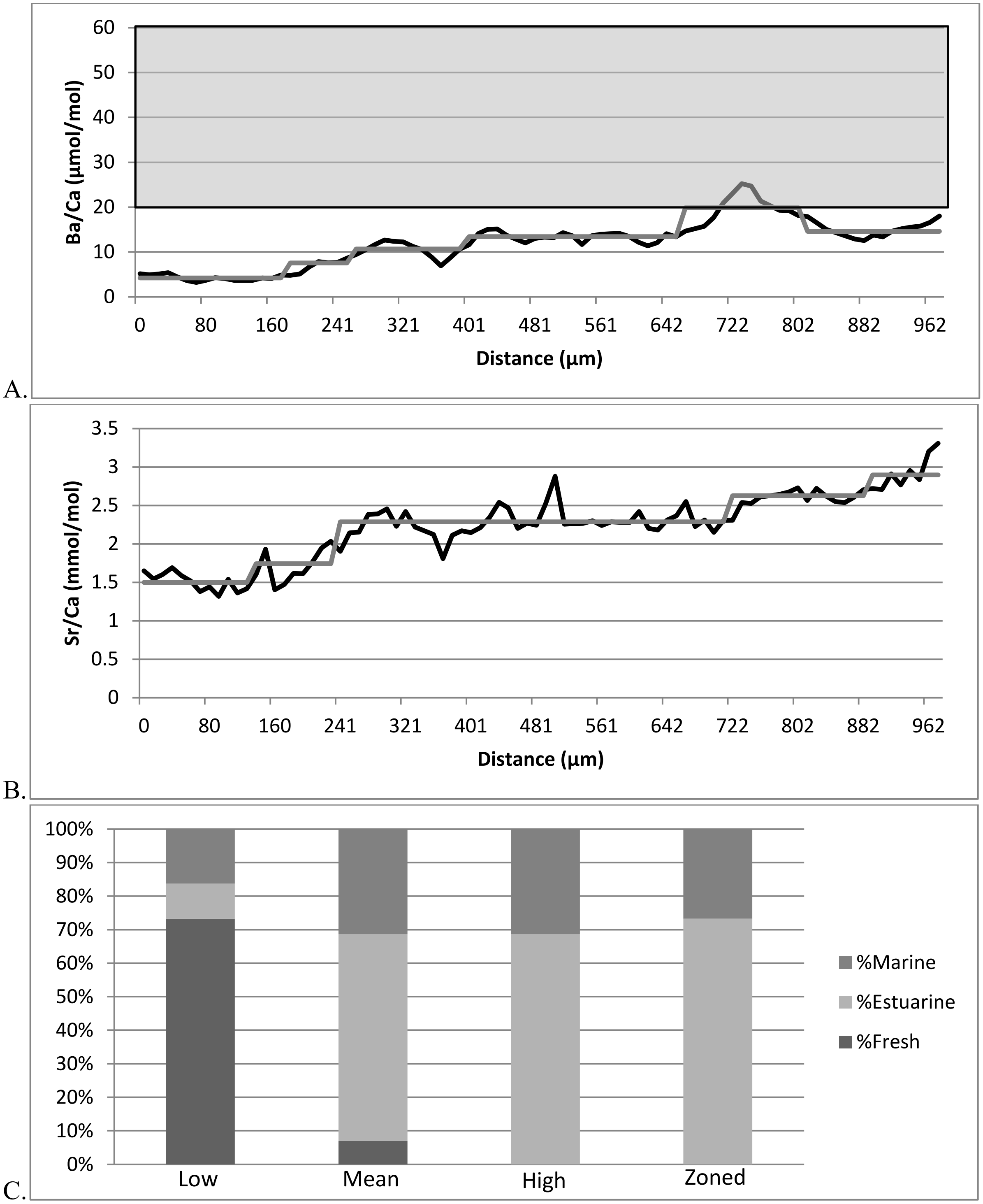
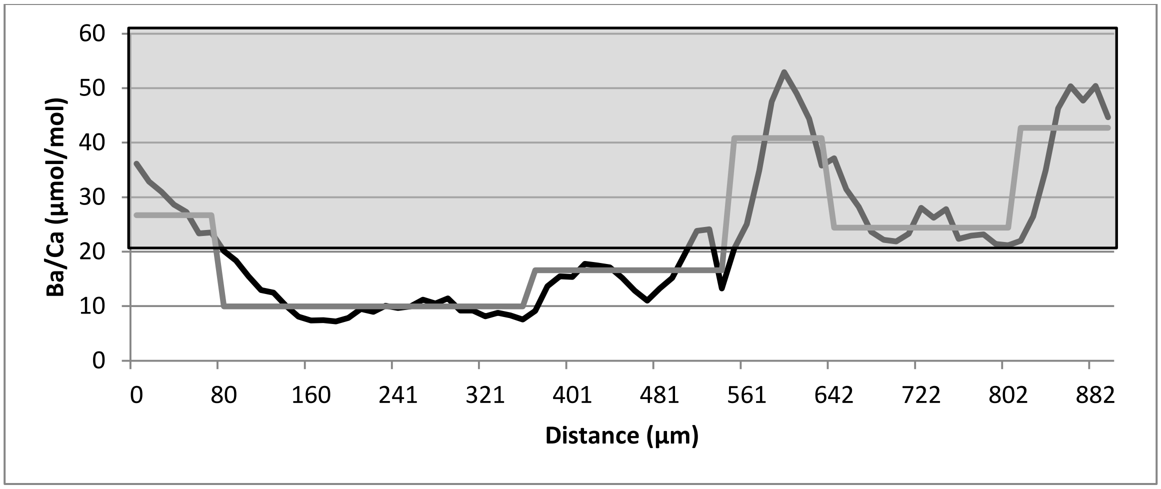
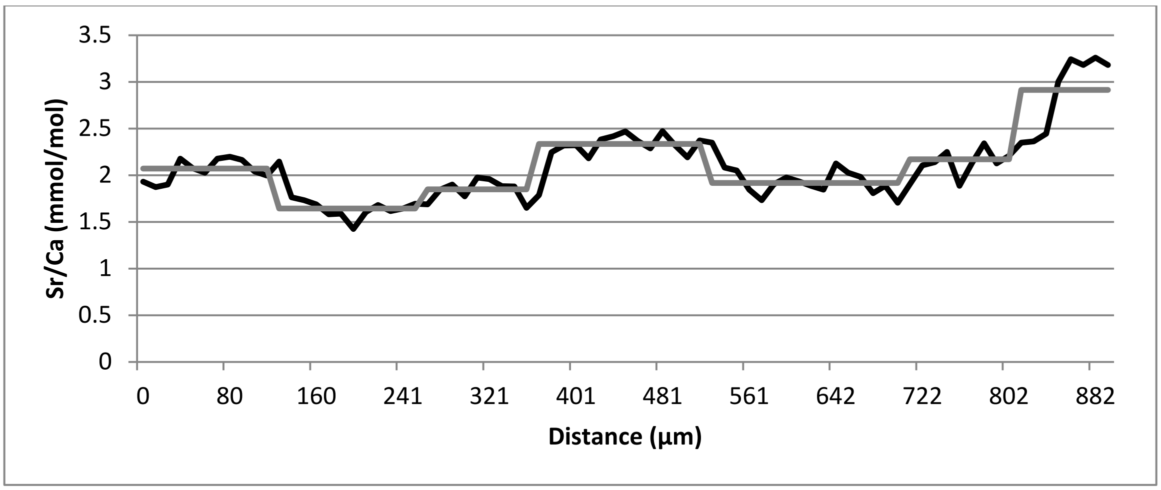


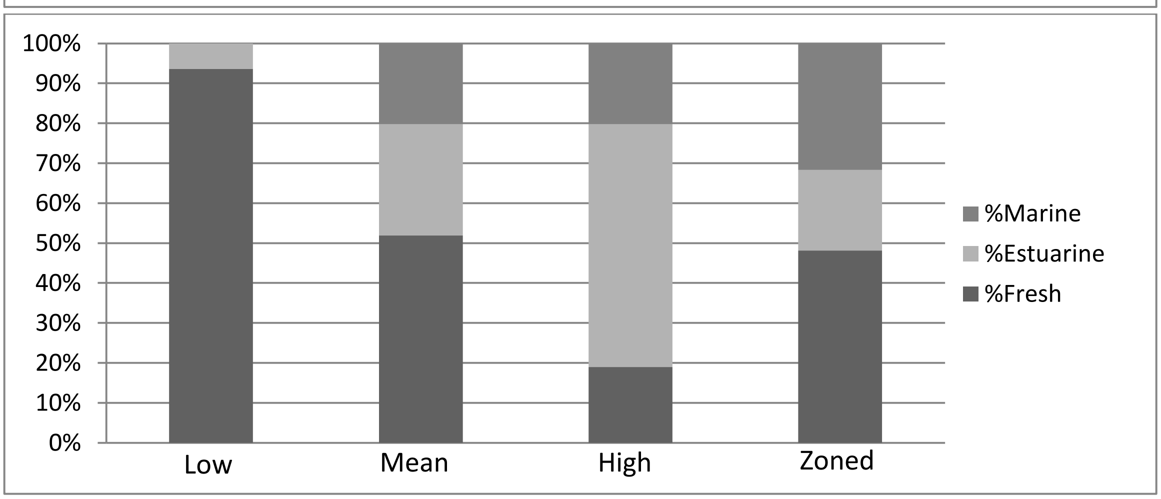
Figure AB.182. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 437. Figure 182.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

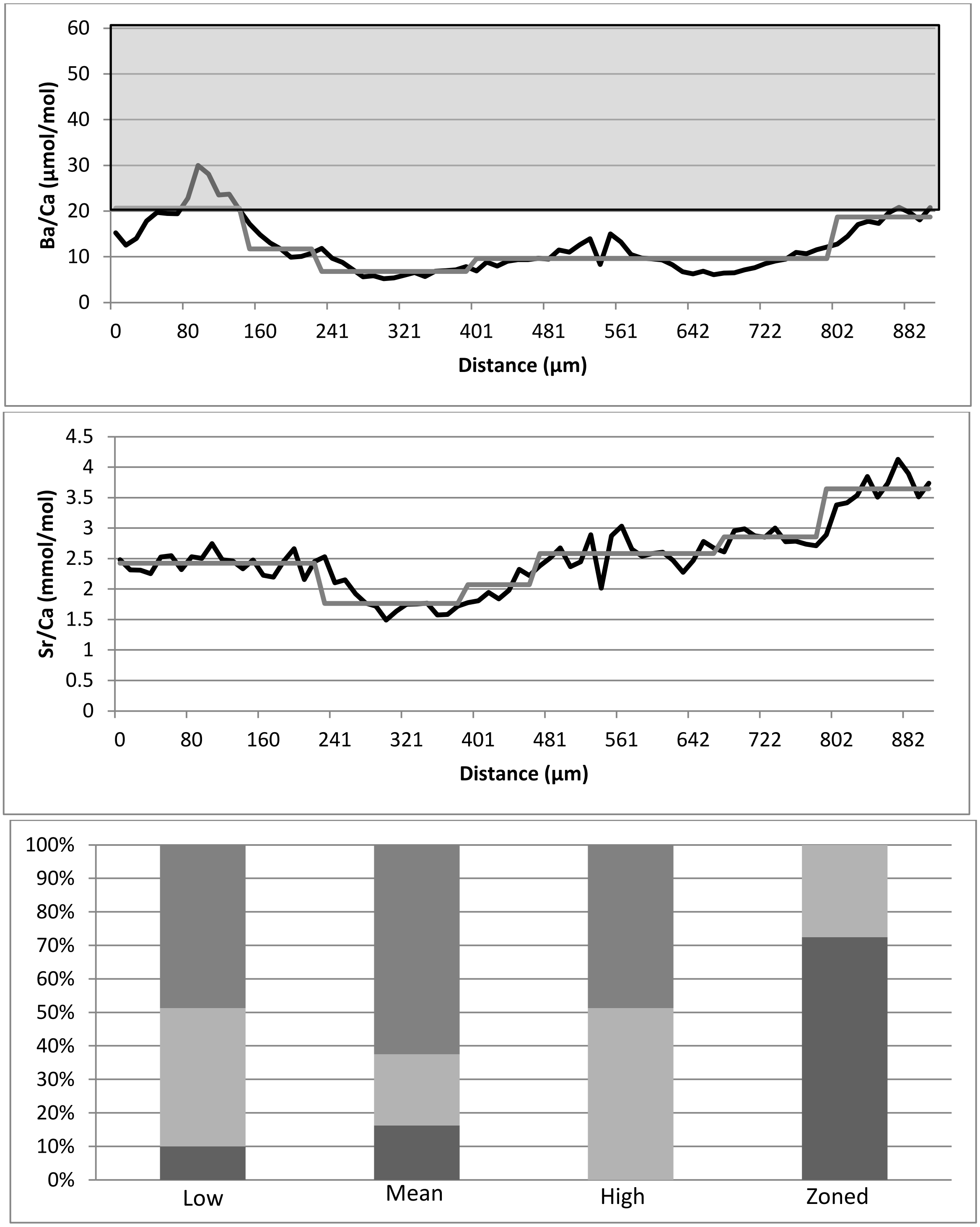


B.



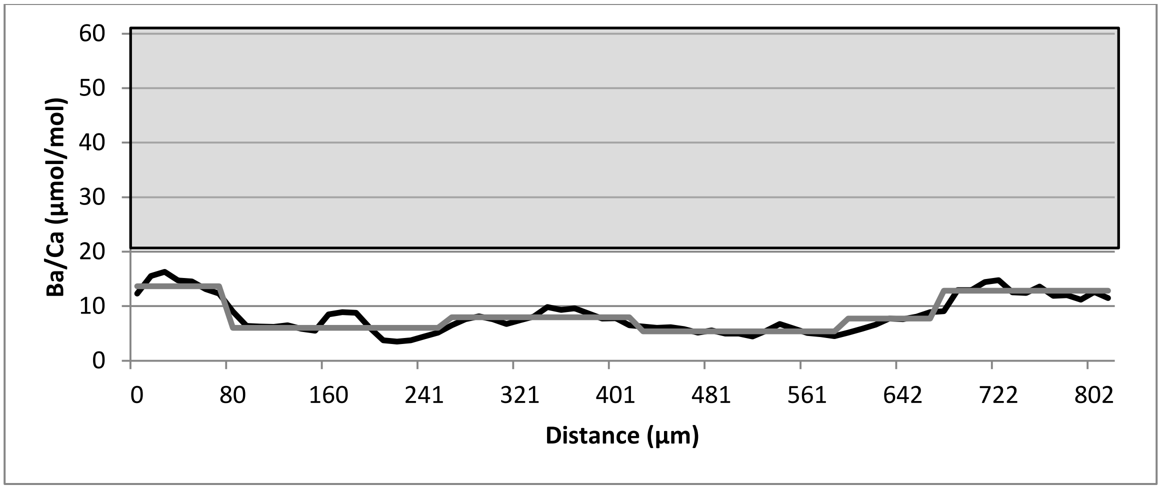
C.

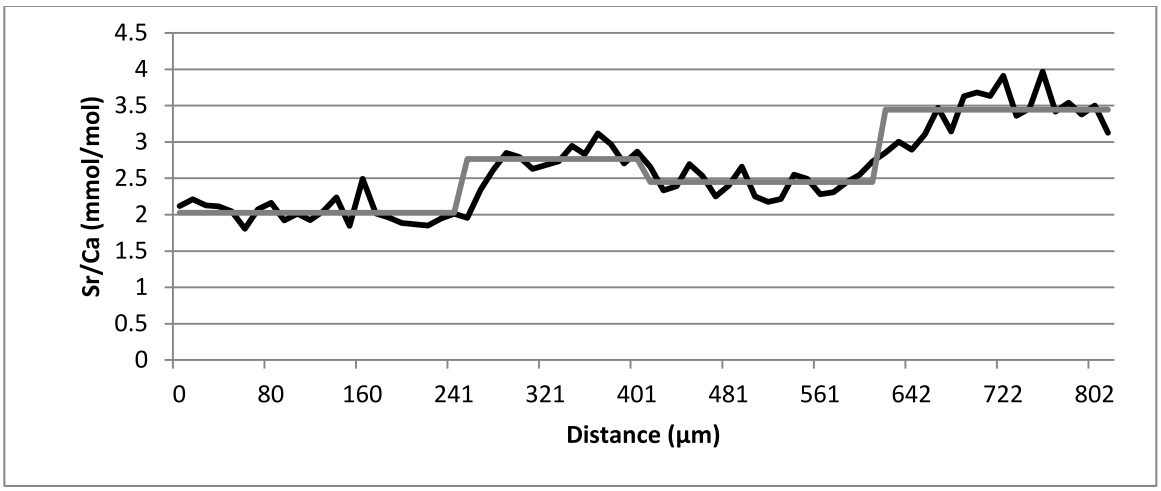
Figure AB.183. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 438. Figure 183.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A. B. C.

Figure AB.184. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 439. Figure 184.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


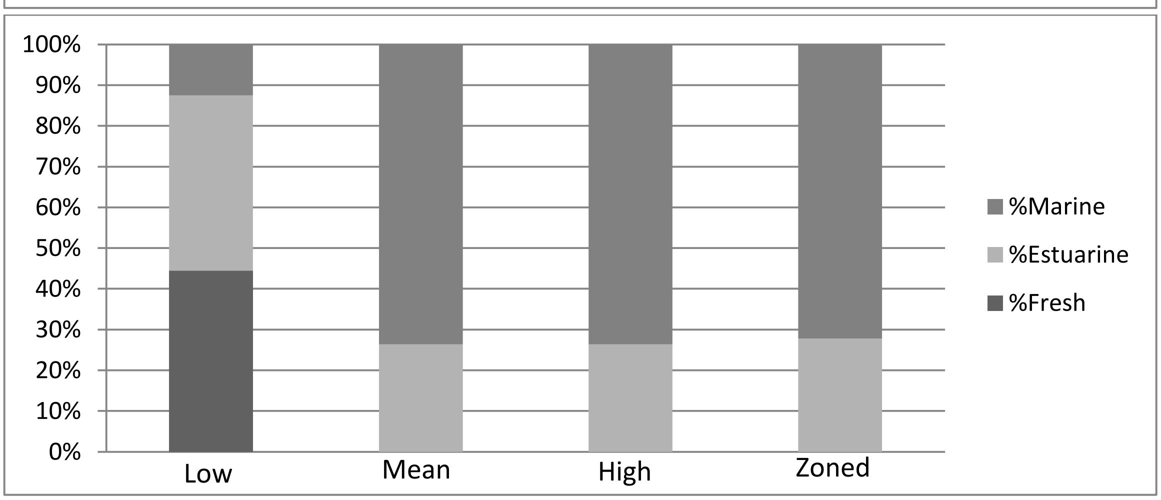
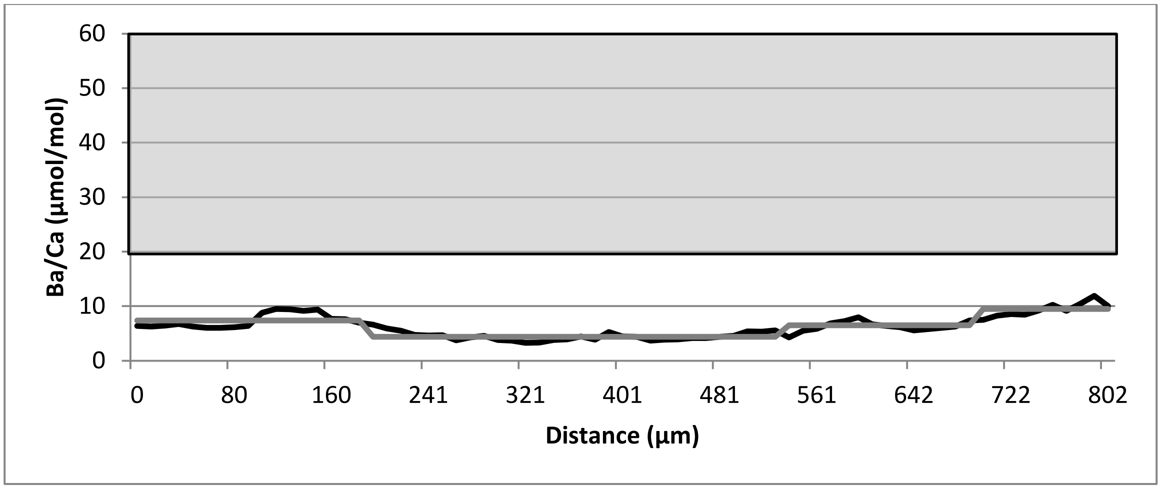
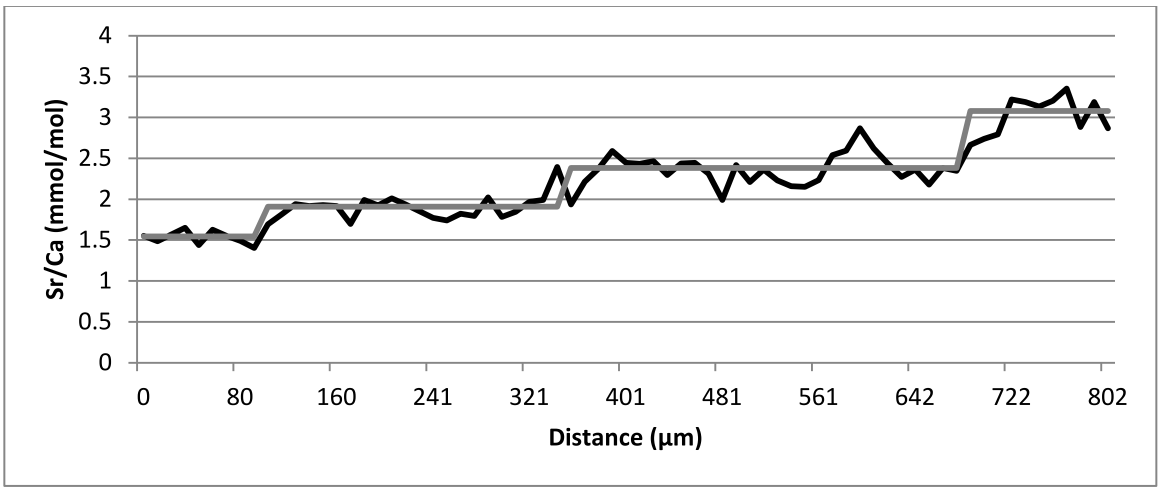
C.


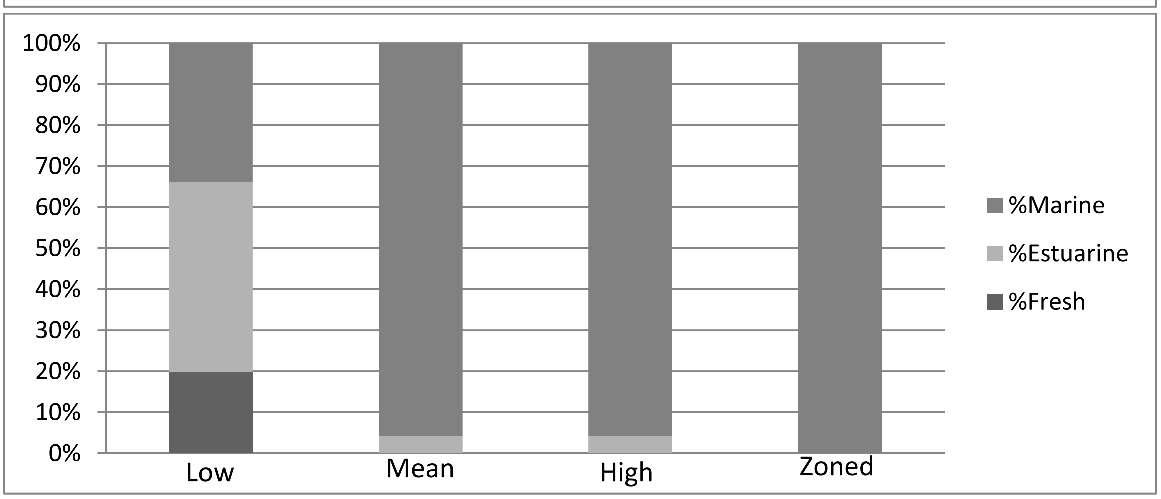
Figure AB.185. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 439. Figure 185.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

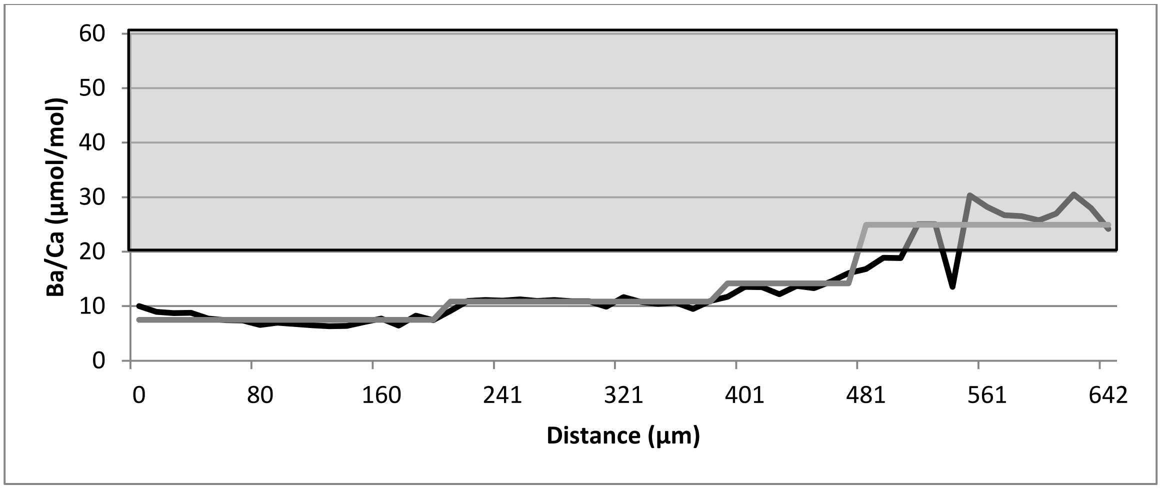


B.

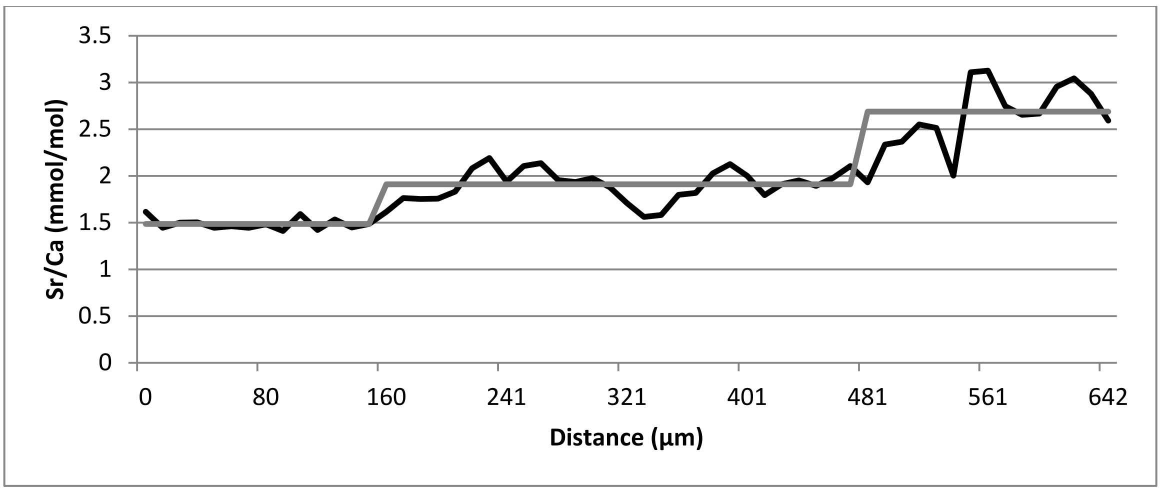


C.

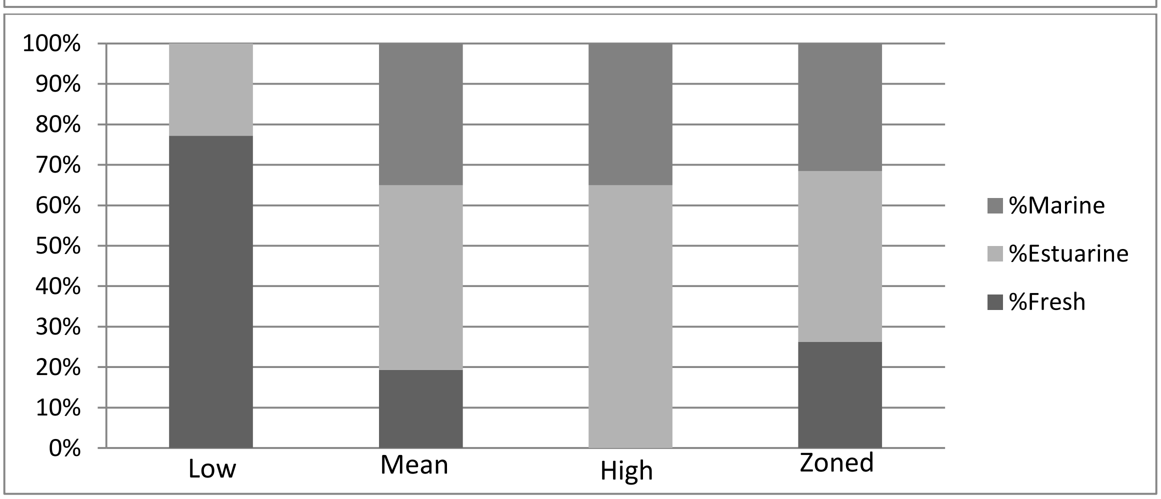
Figure AB.186. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 446. Figure 186.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

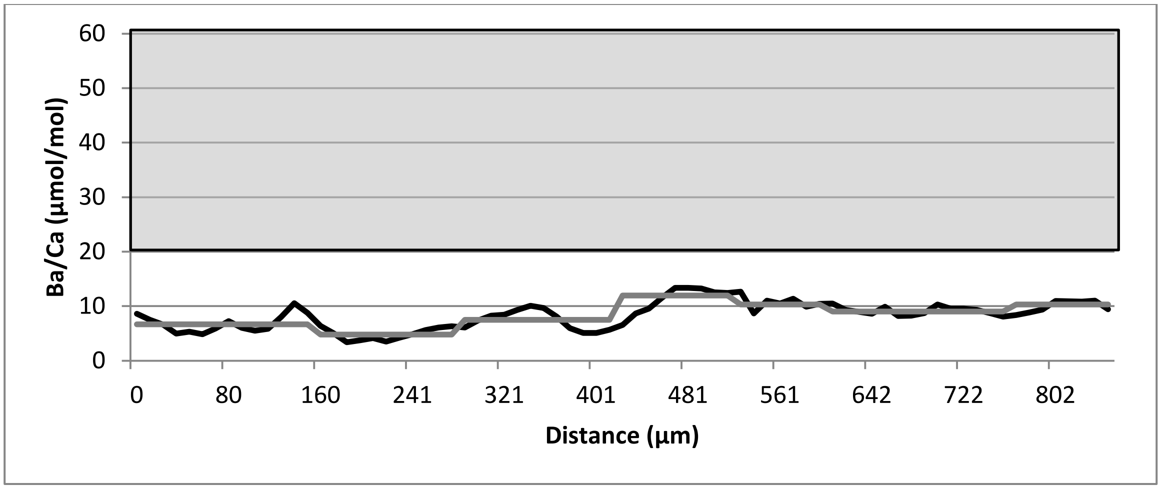


B.

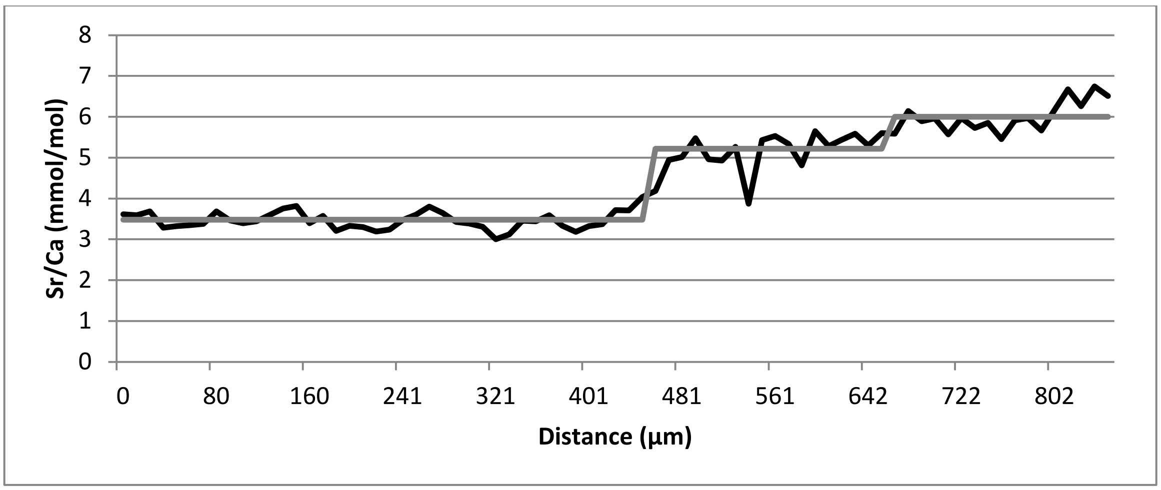


C.

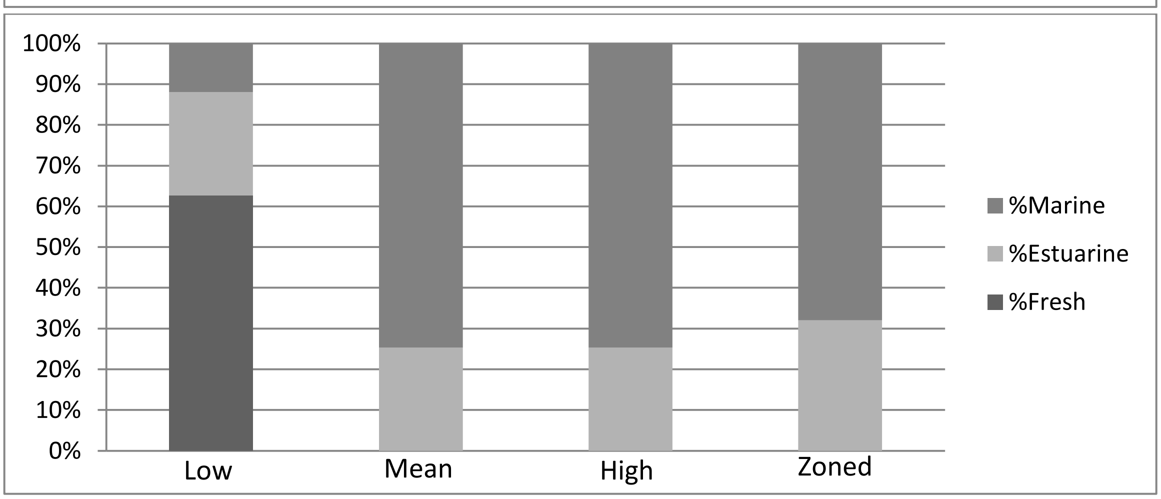
Figure AB.187. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 449. Figure 187.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

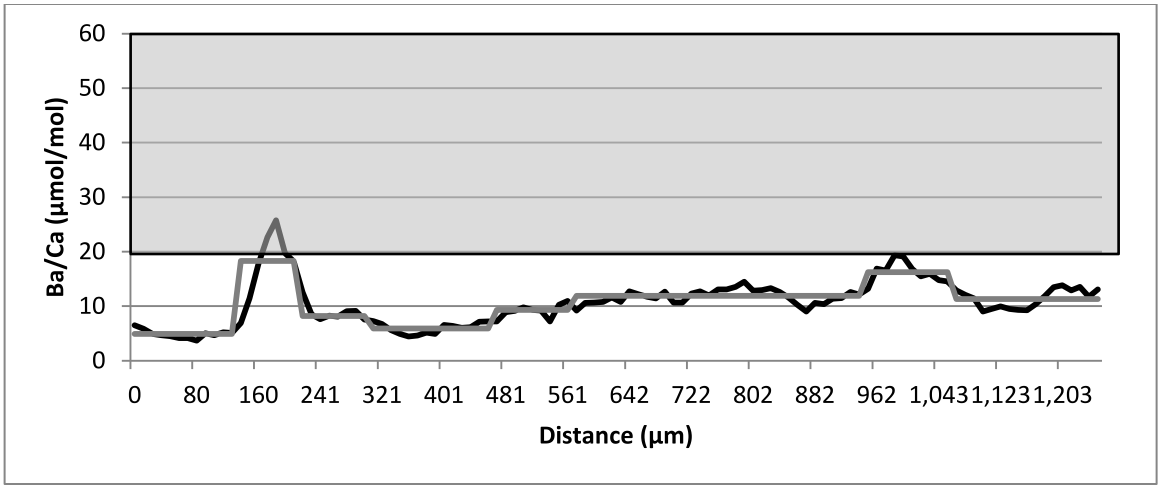


B.

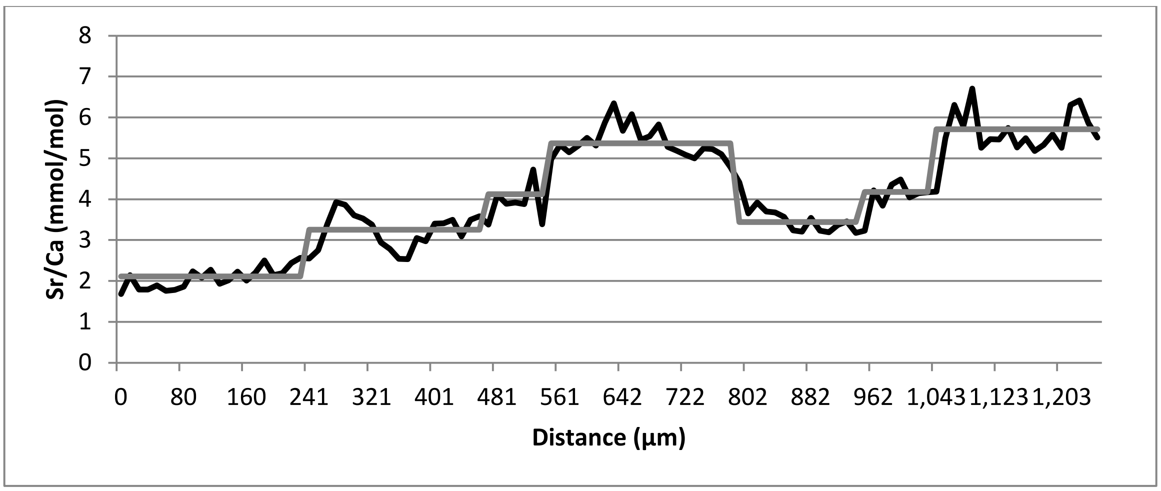


C.

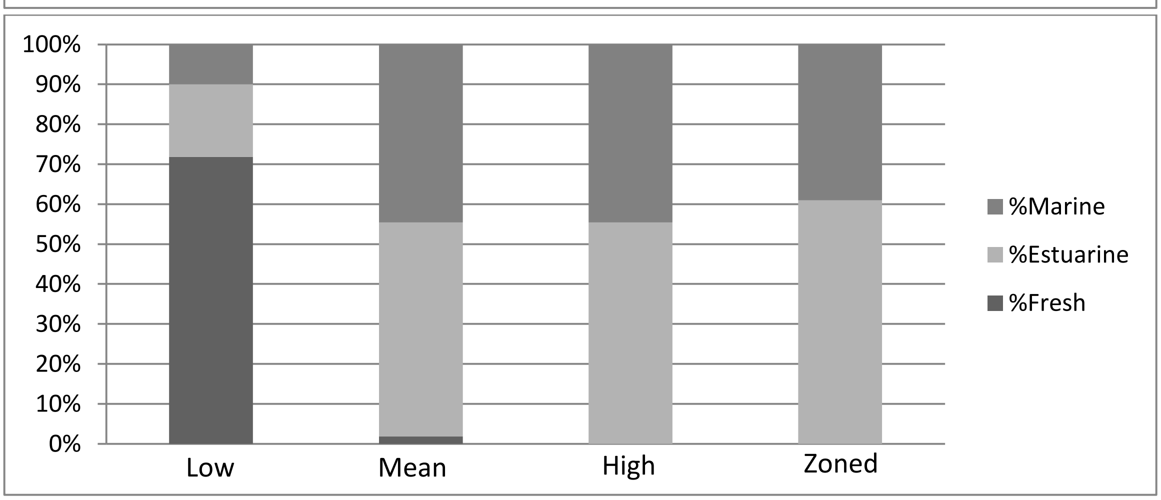
Figure AB.188. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 450. Figure 188.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

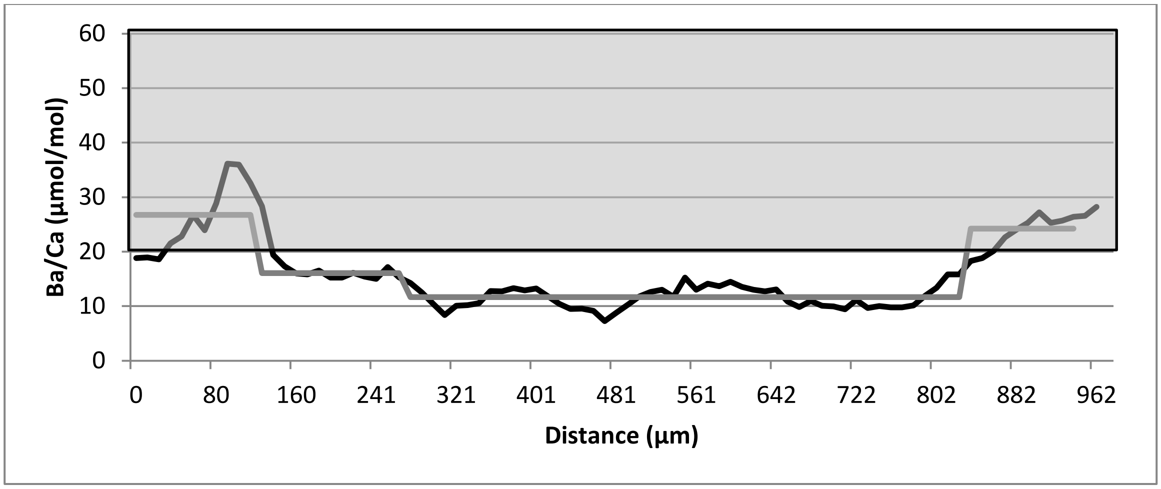


B.

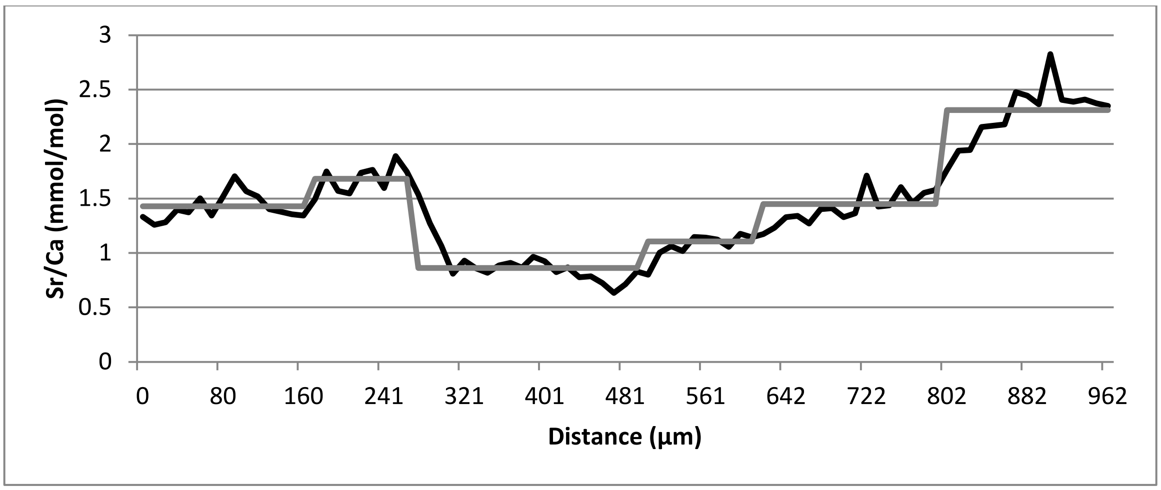


C.

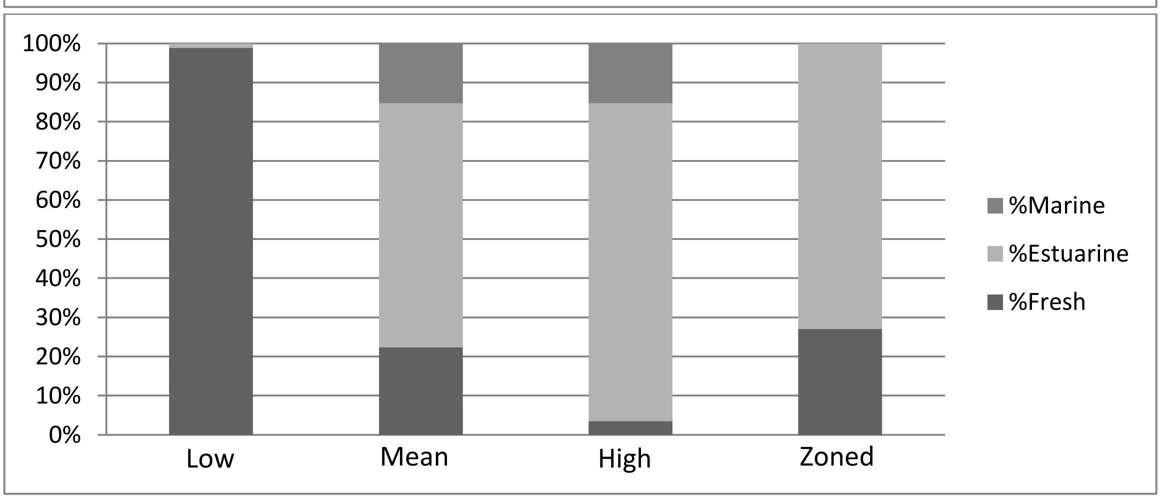
Figure AB.189. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 451. Figure 189.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.190. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 454. Figure 190.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

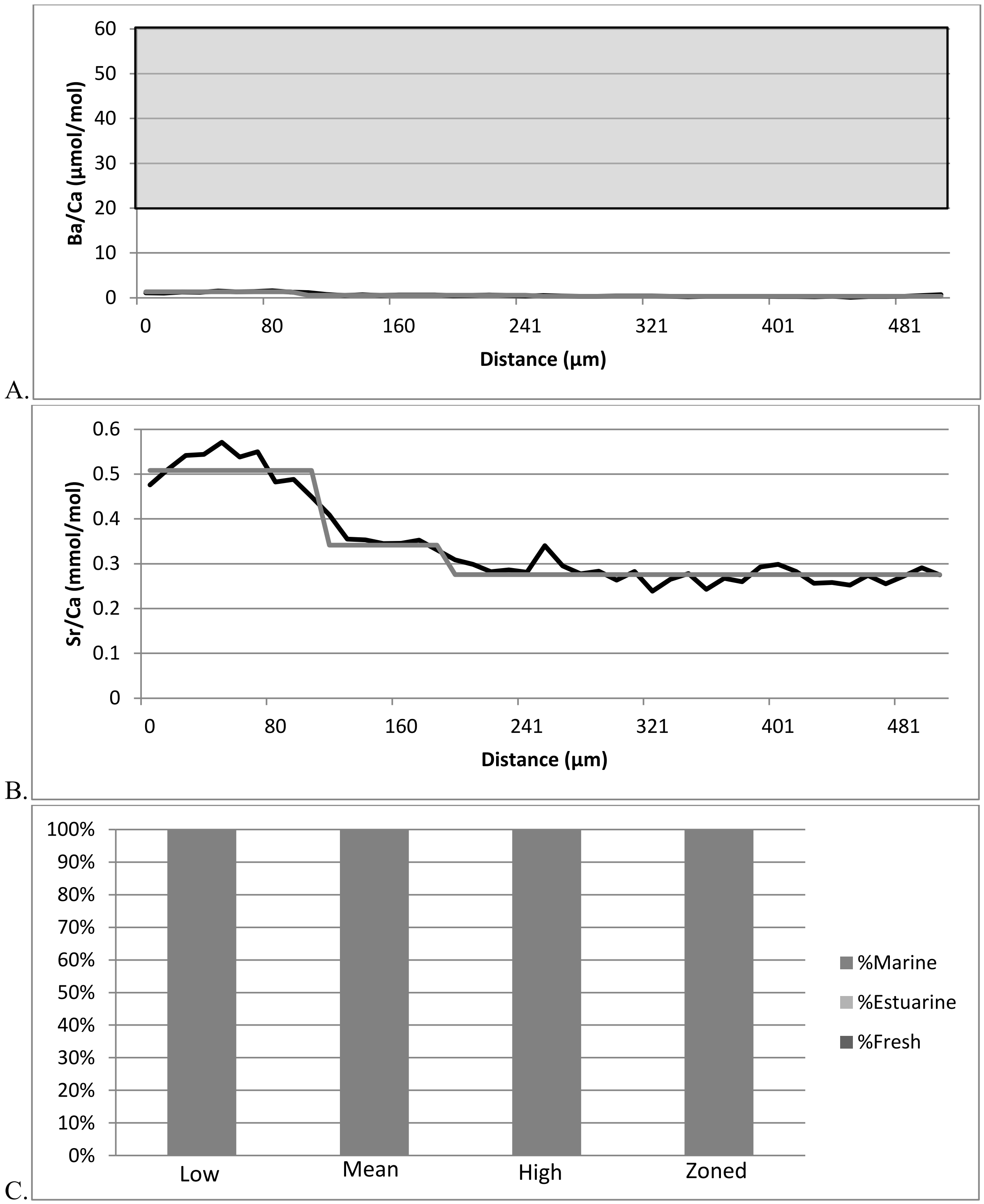
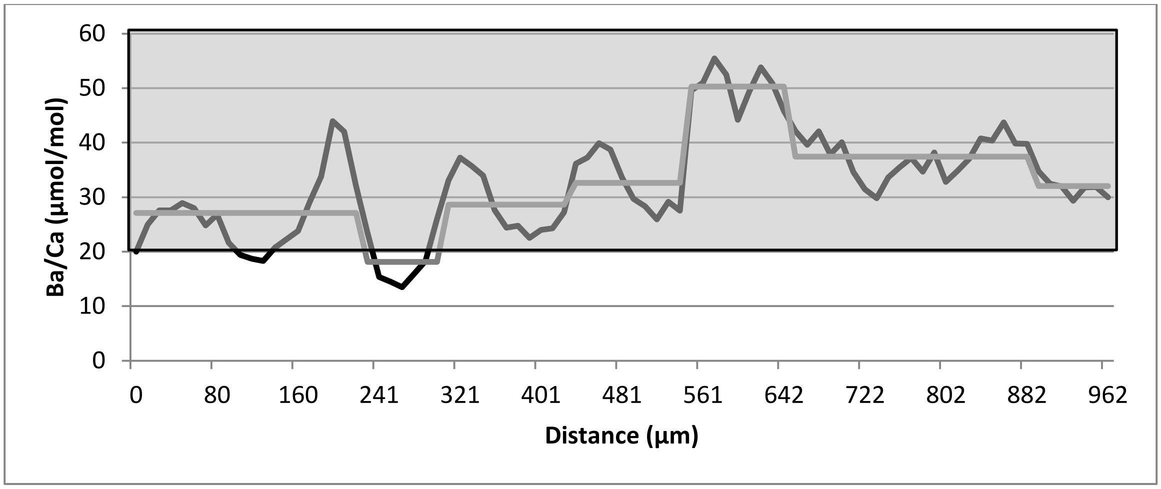
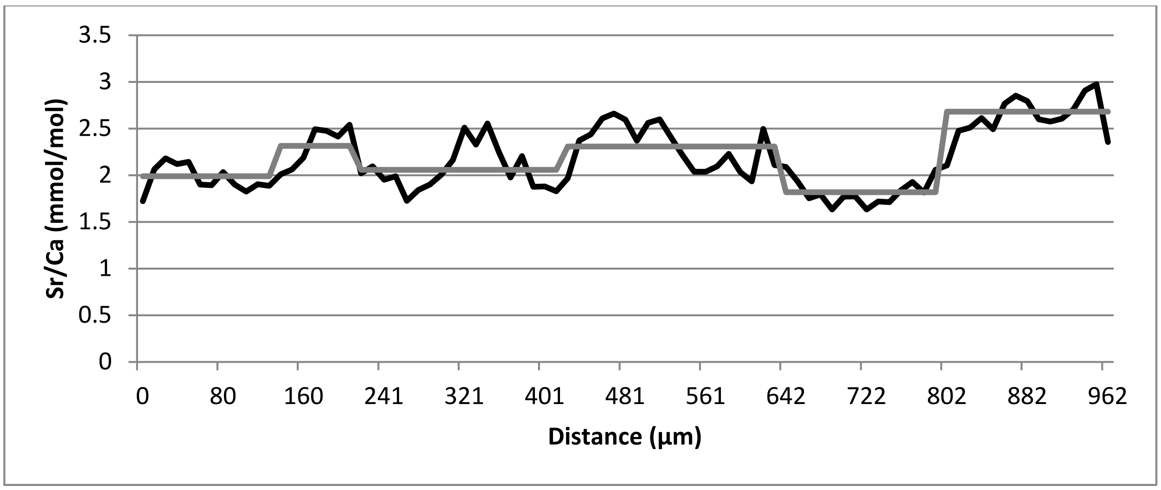


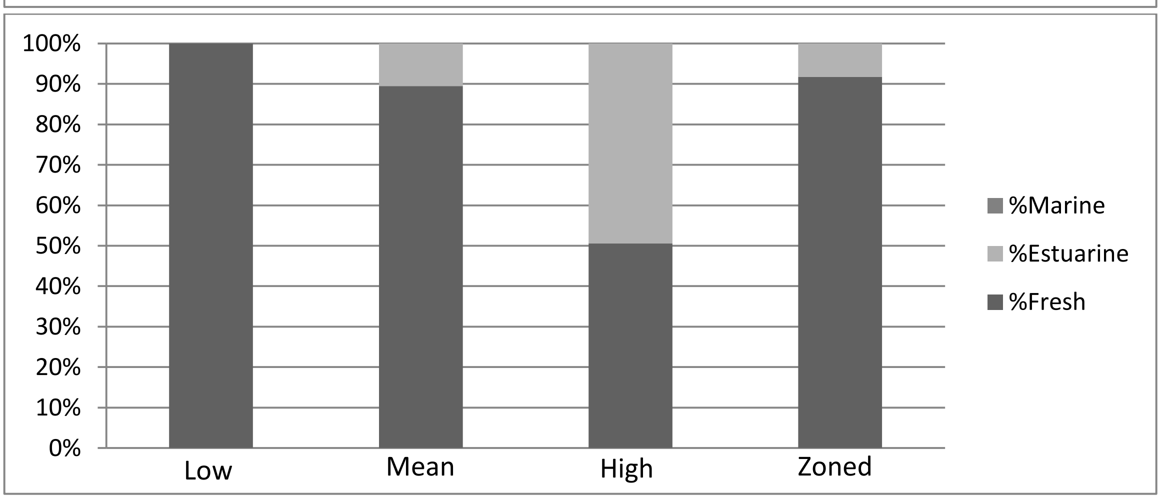
Figure AB.191. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 457. Figure 191.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

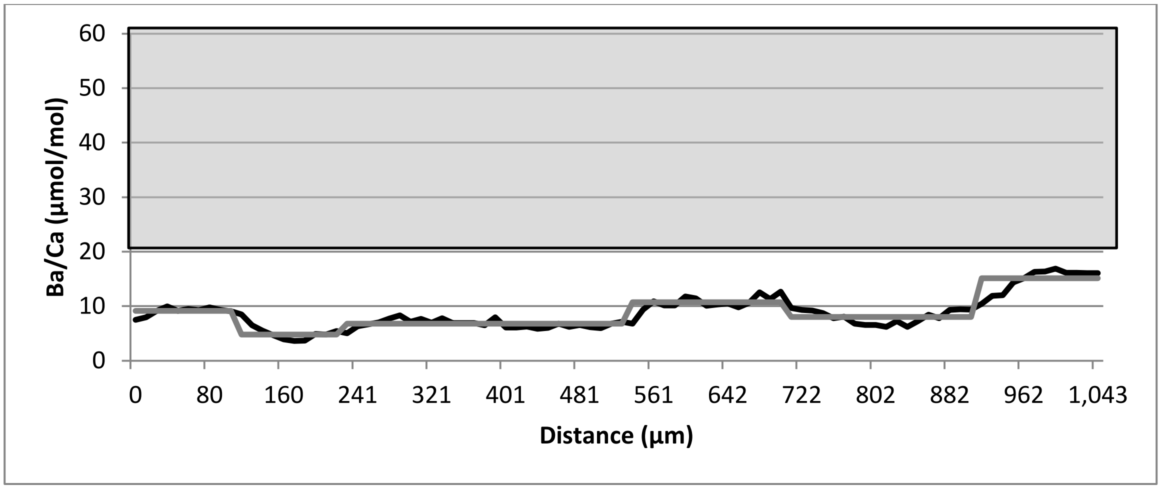


B.

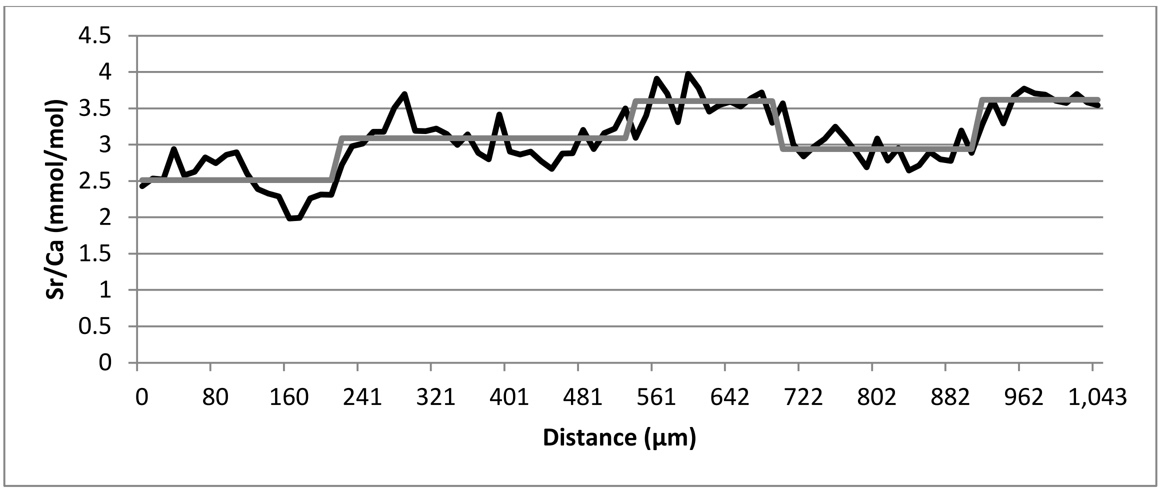


C.

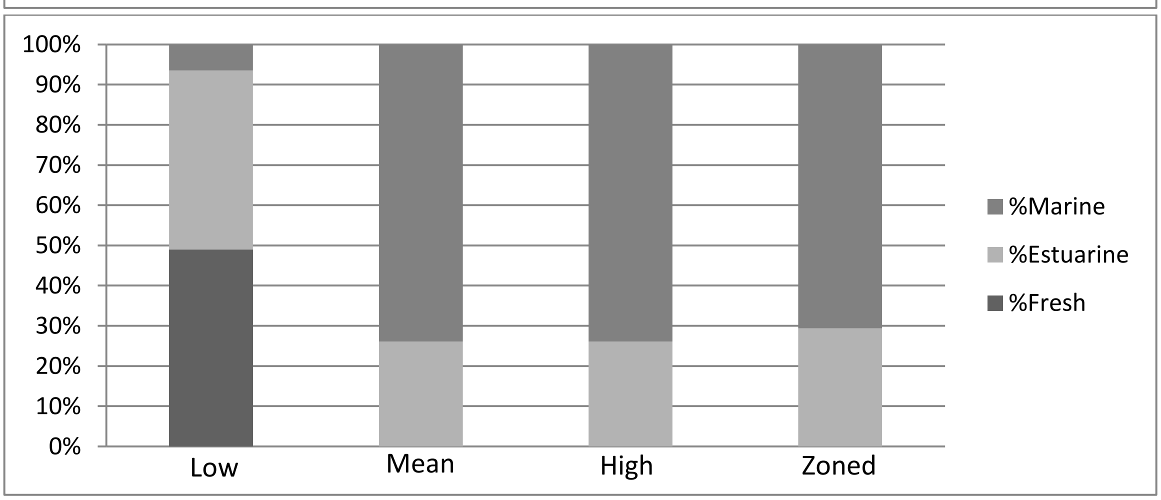
Figure AB.192. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 458. Figure 192.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

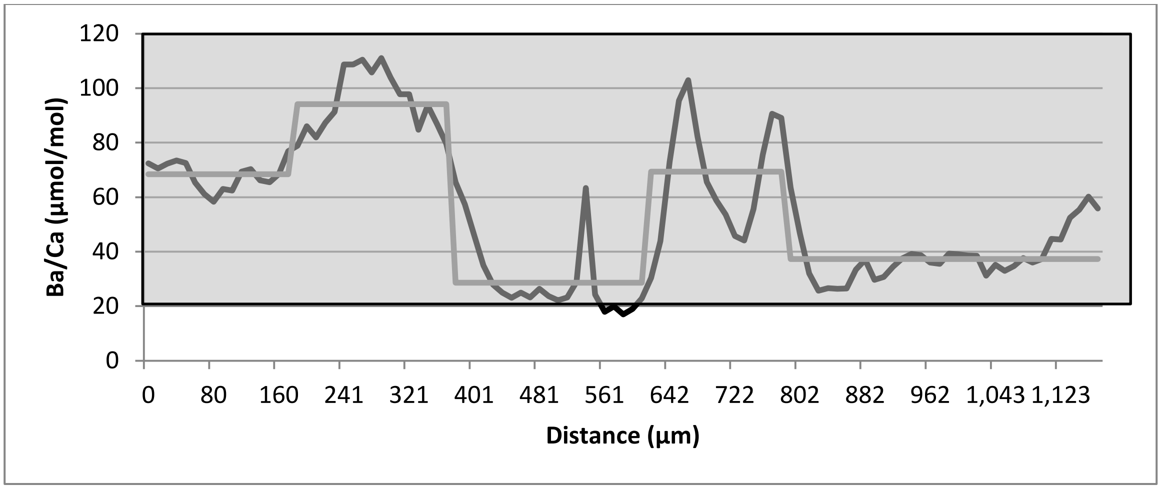


B.

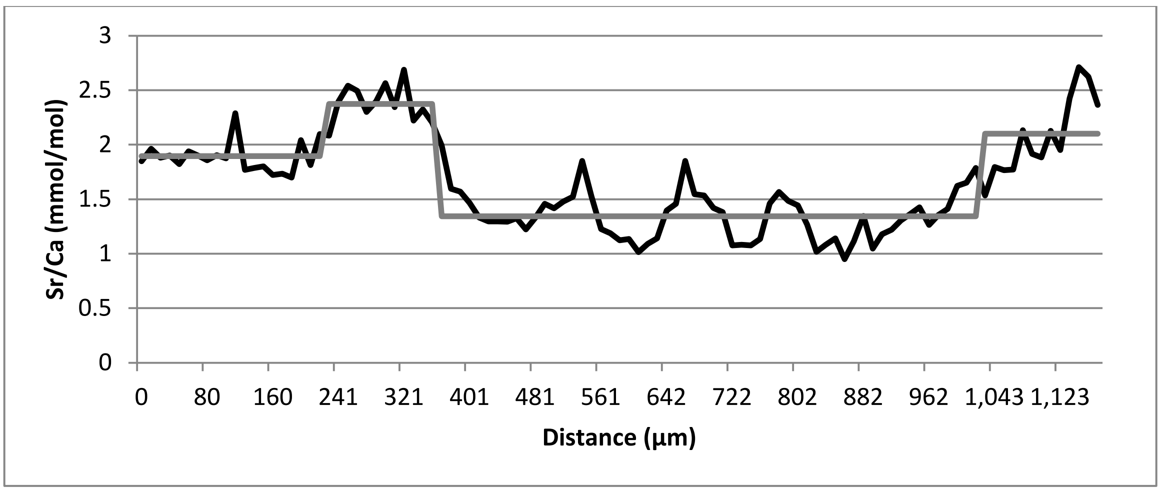


C.

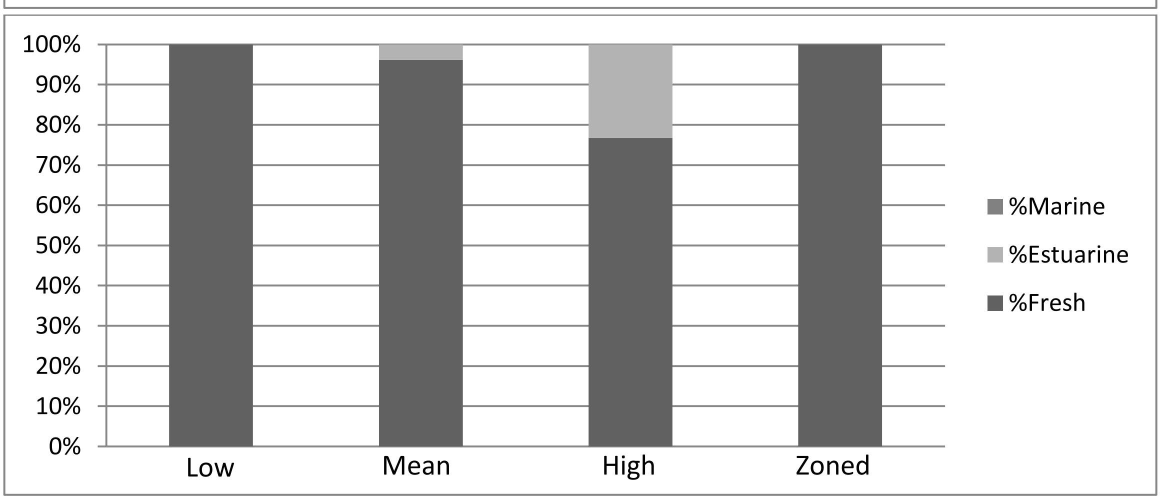
Figure AB.193. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 459. Figure 193.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

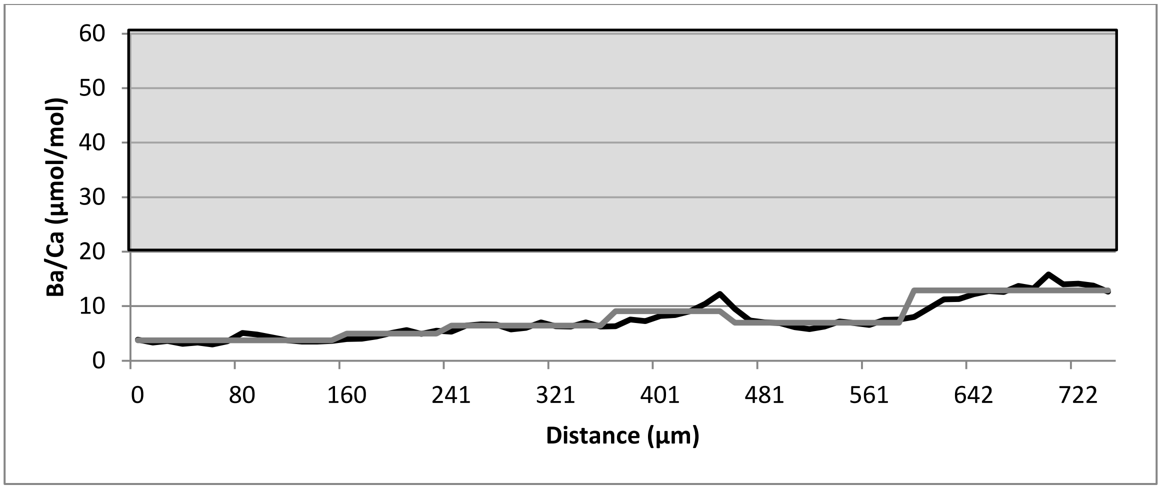


B.

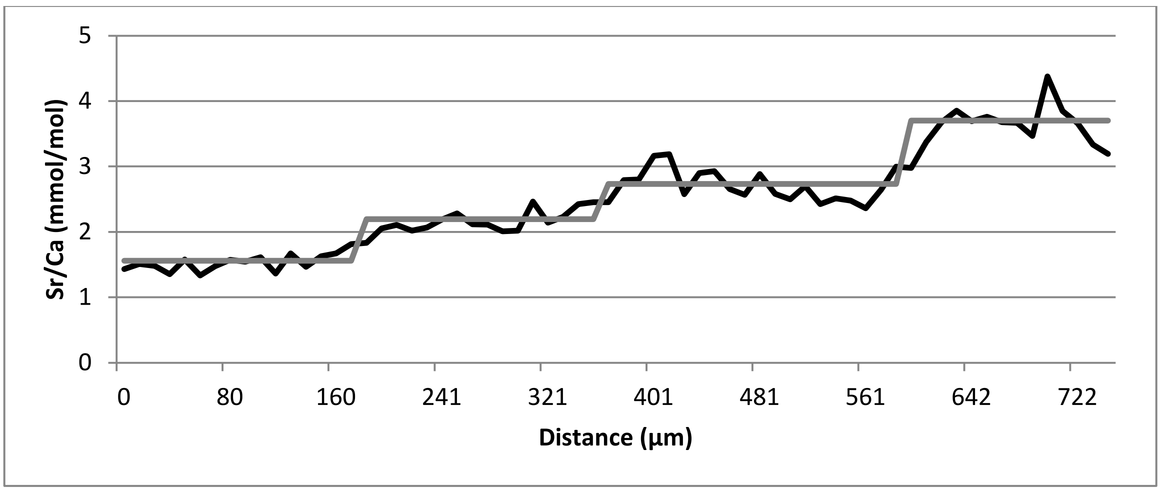


C.

Figure AB.194. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 461. Figure 194.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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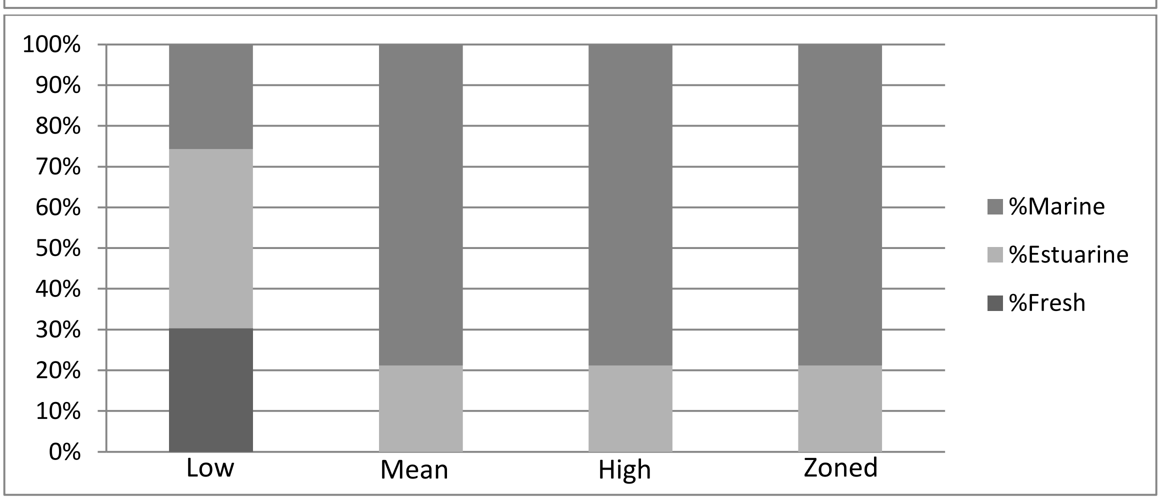


C.

Figure AB.195. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 462. Figure 195.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

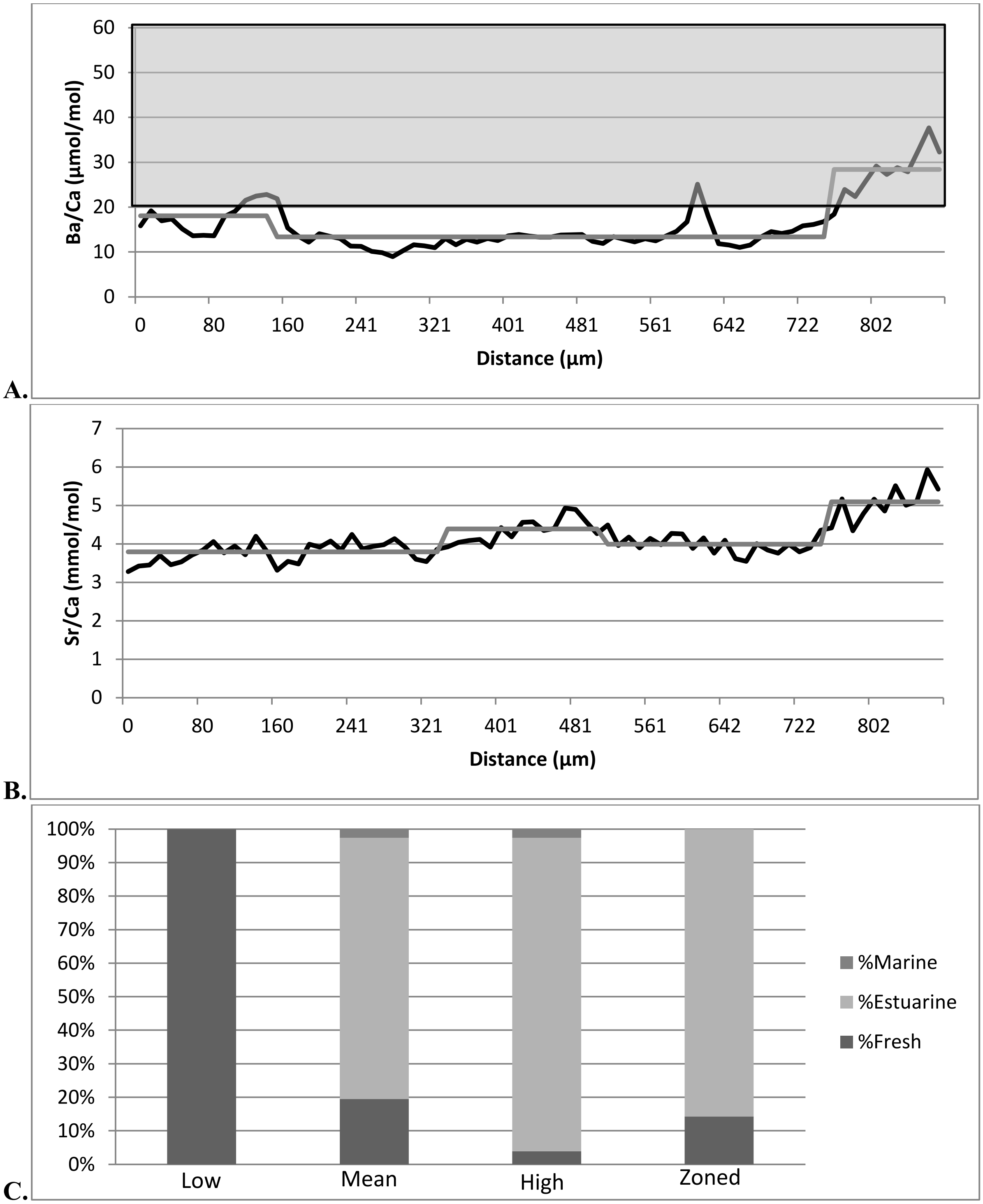
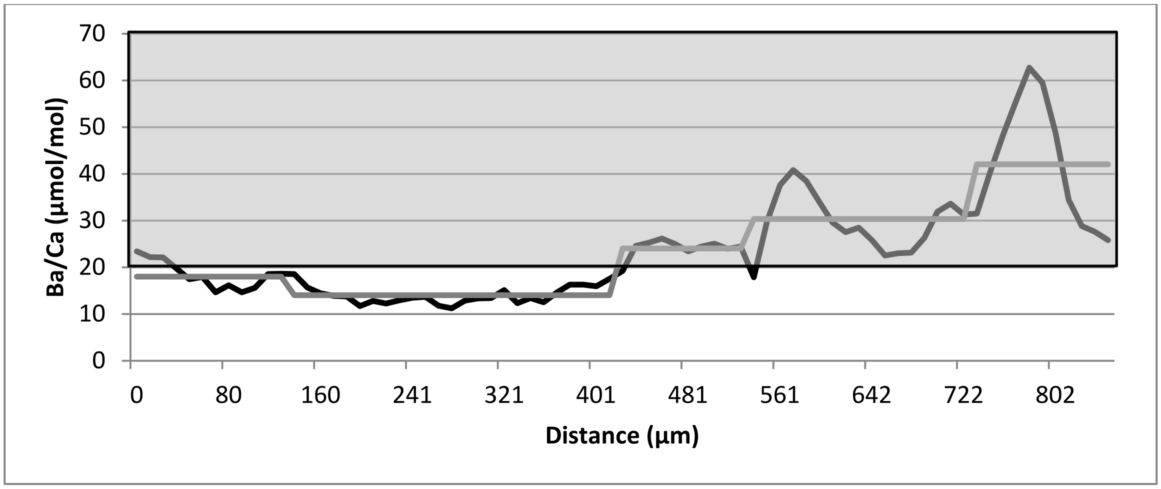
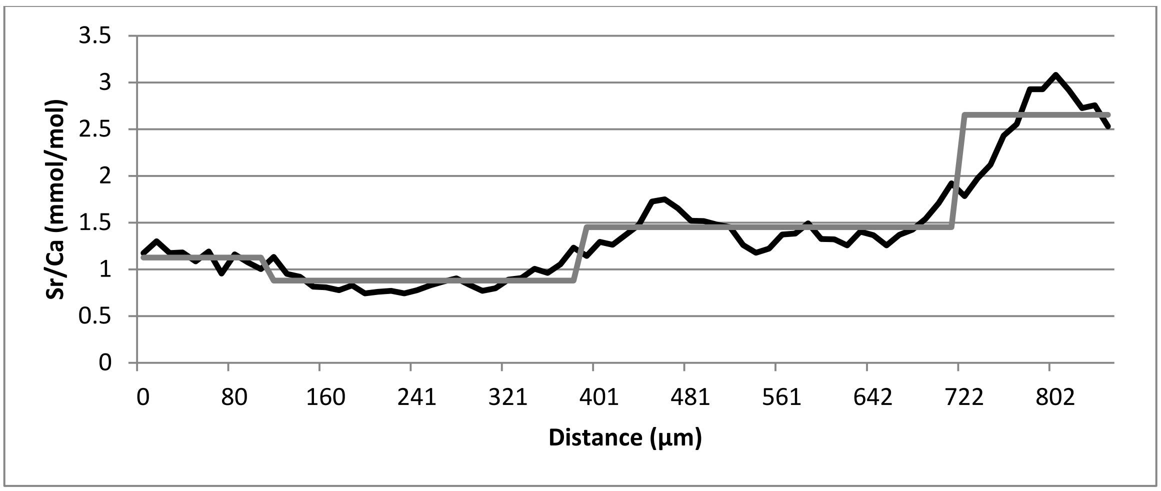


Figure AB.196. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 463. Figure 196.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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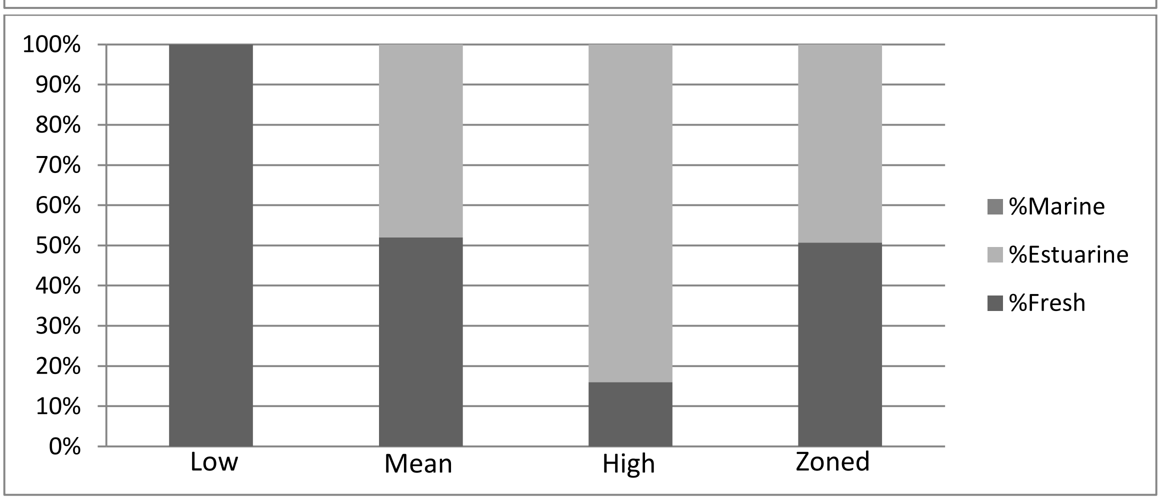
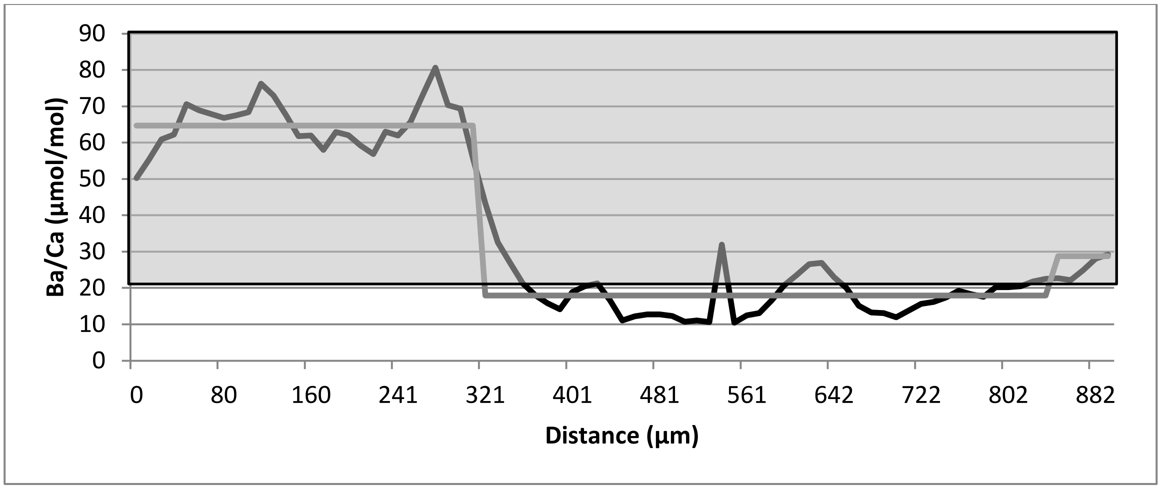
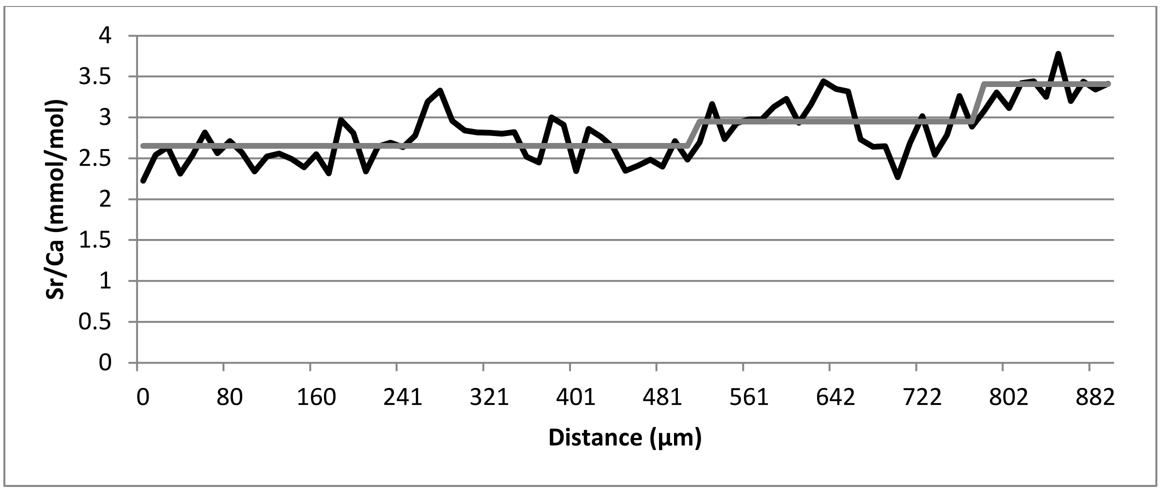


C.

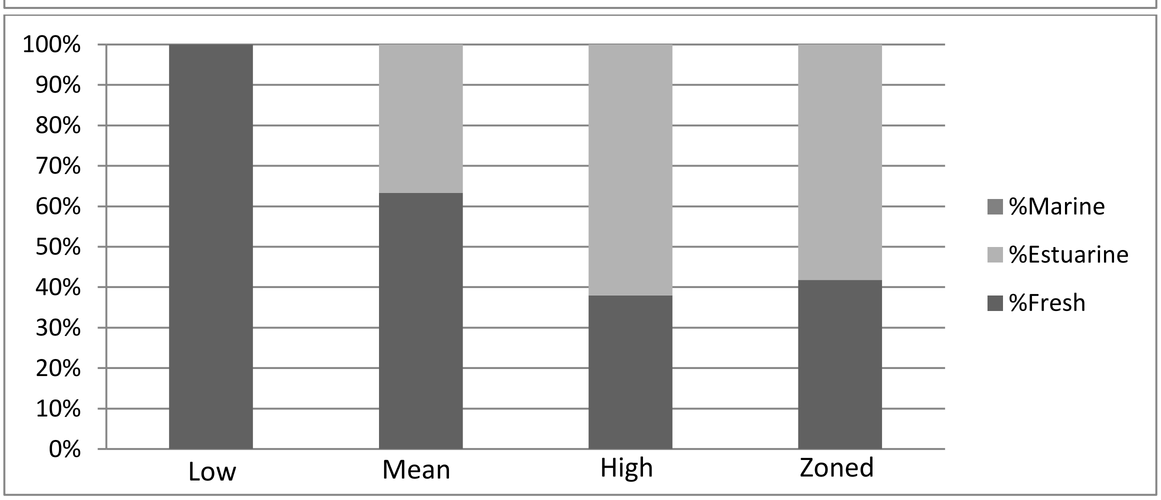
Figure AB.197. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 465. Figure 197.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

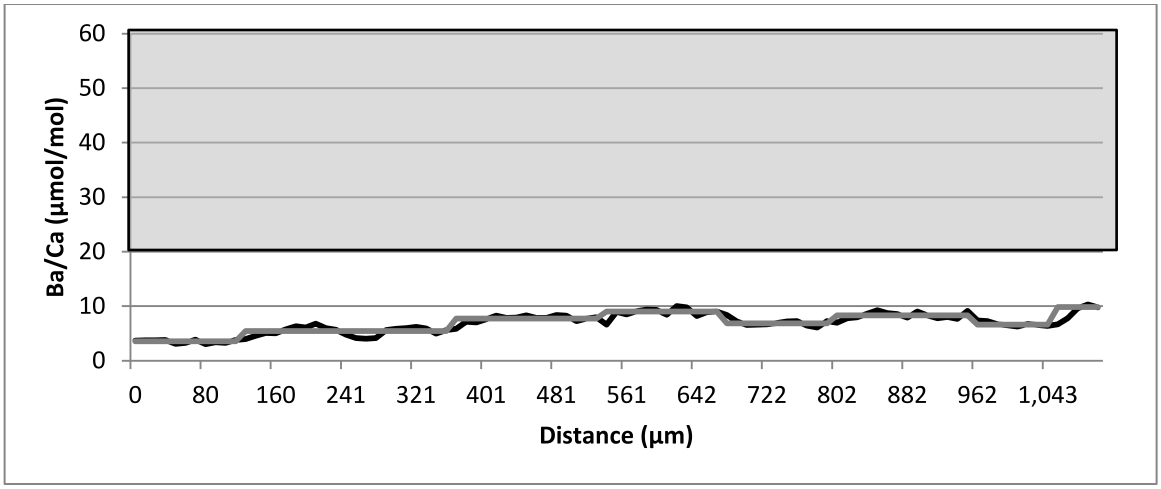


B.

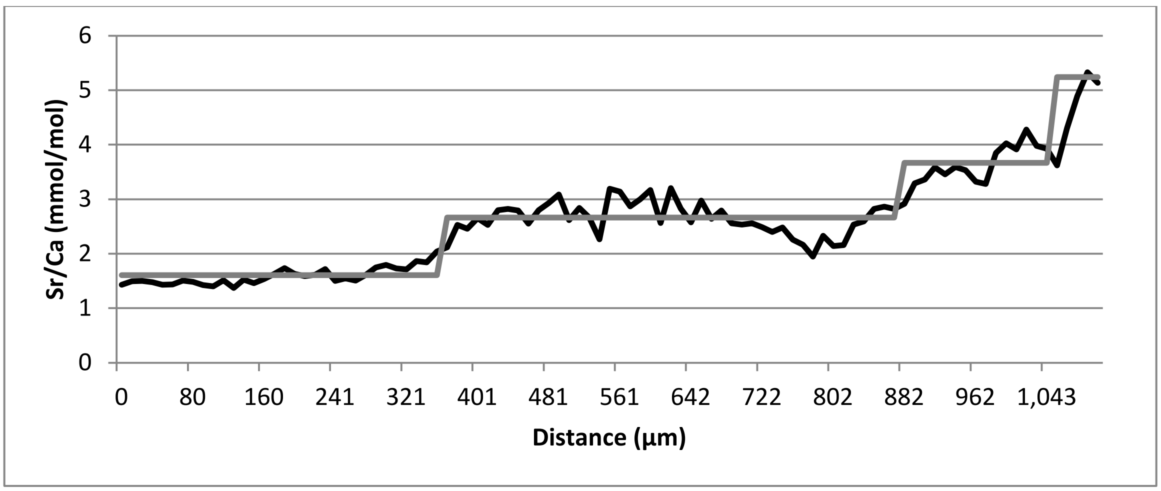


C.

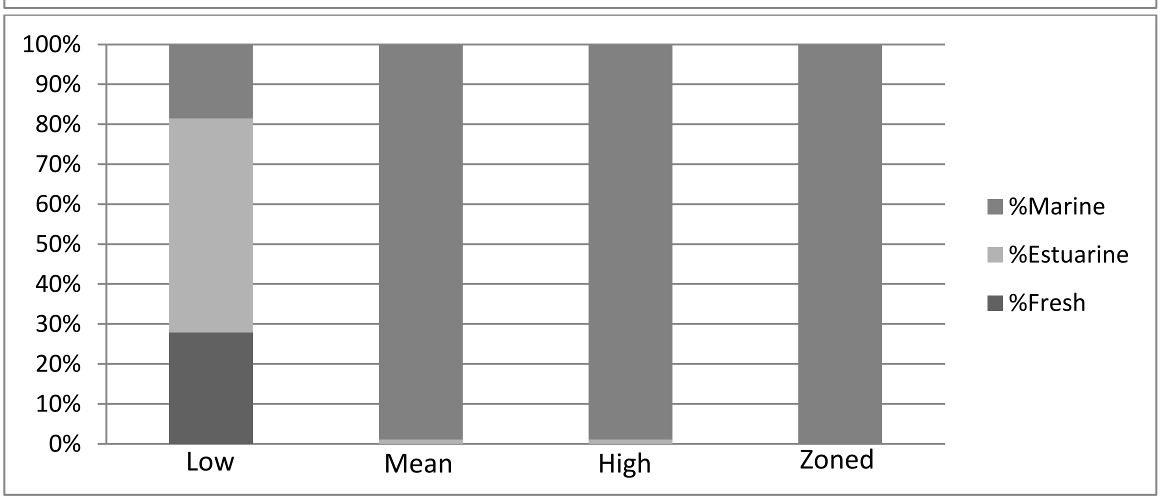
Figure AB.198. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 467. Figure 198.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.199. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 468. Figure 199.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

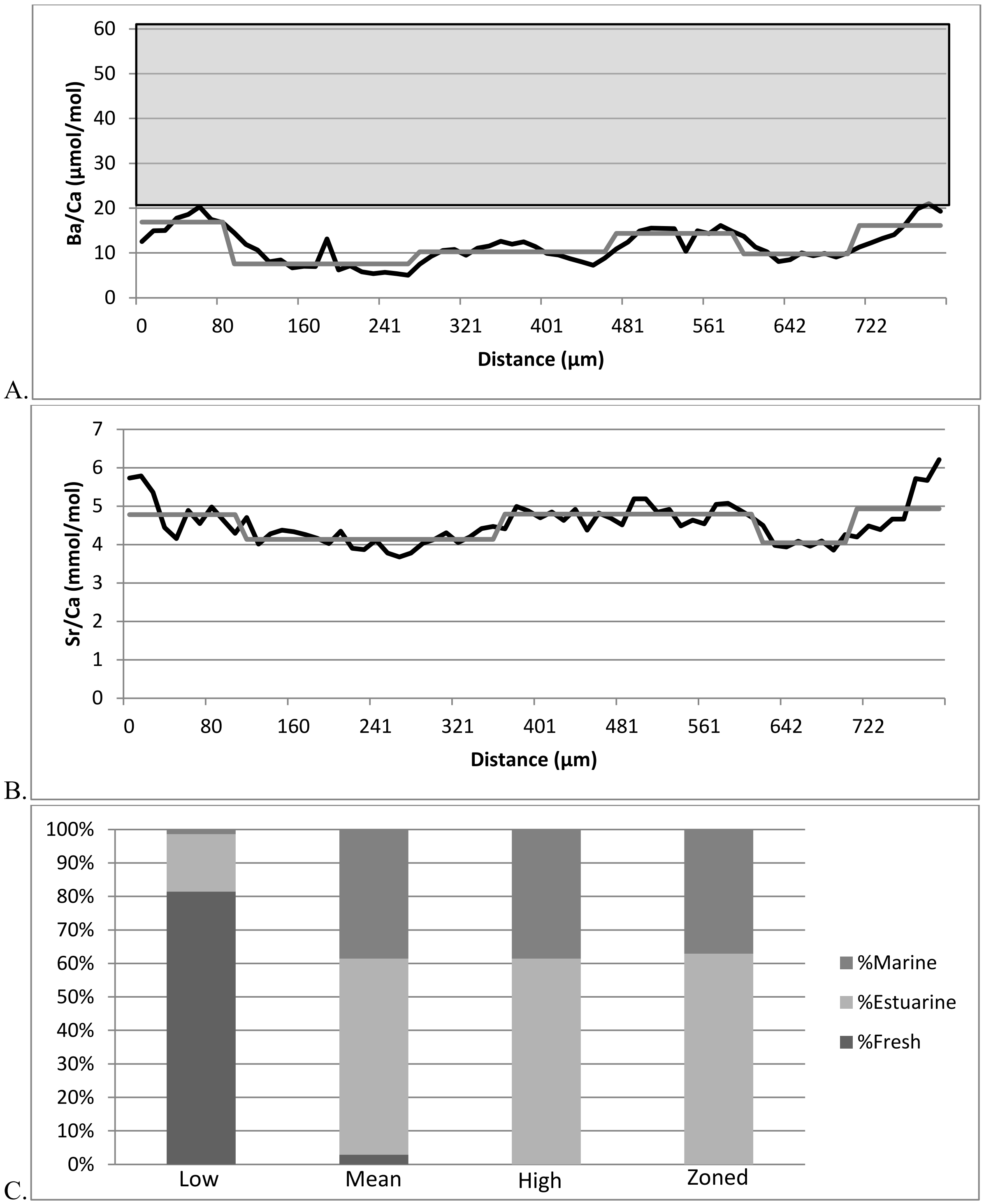
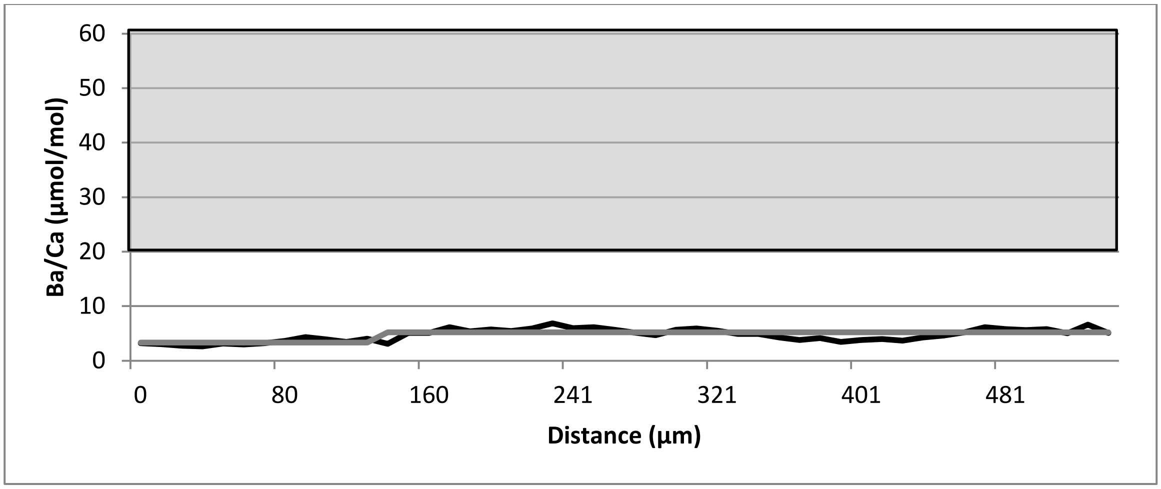
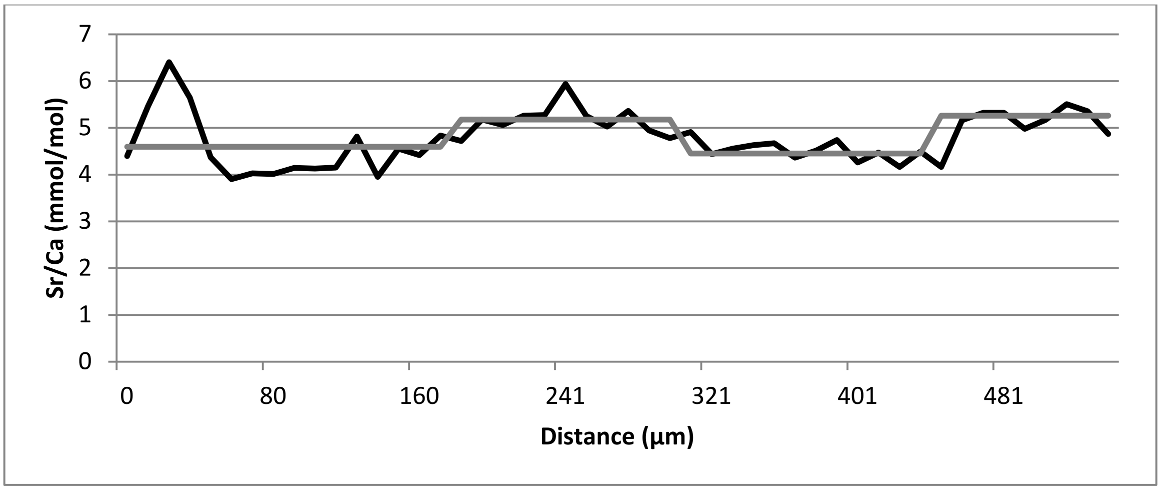


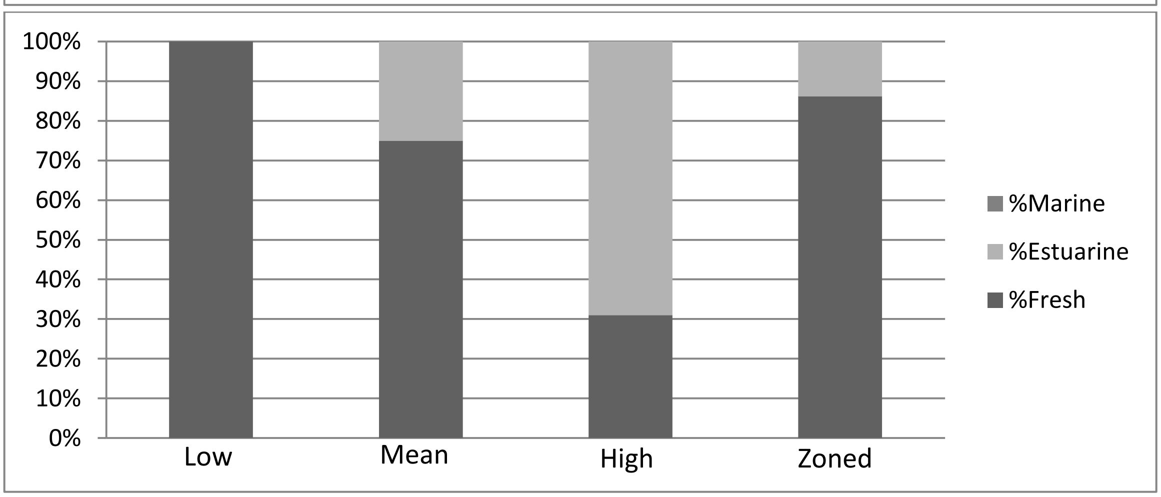
Figure AB.200. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 468.1. Figure 200.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.201. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 503. Figure 201.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

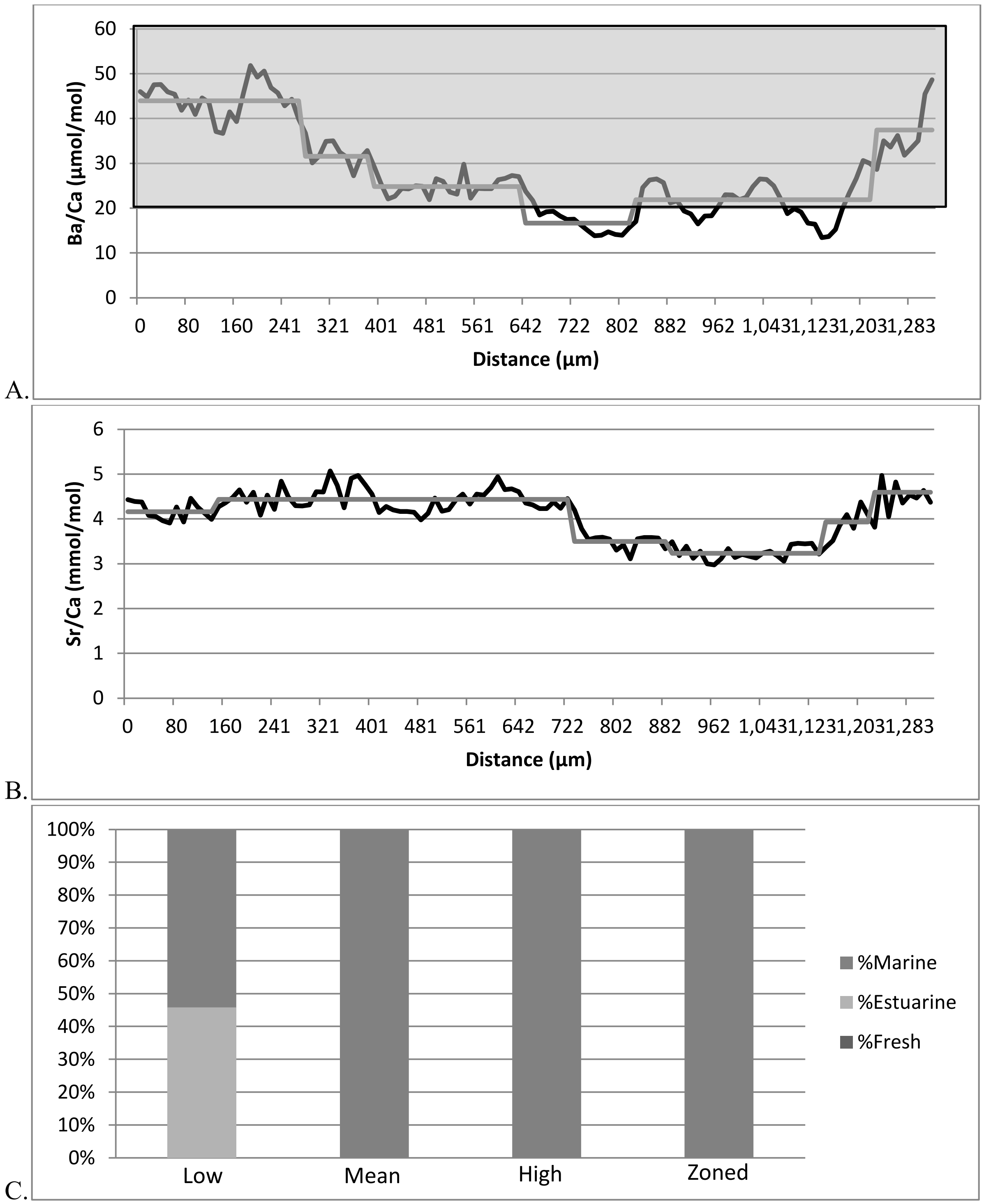
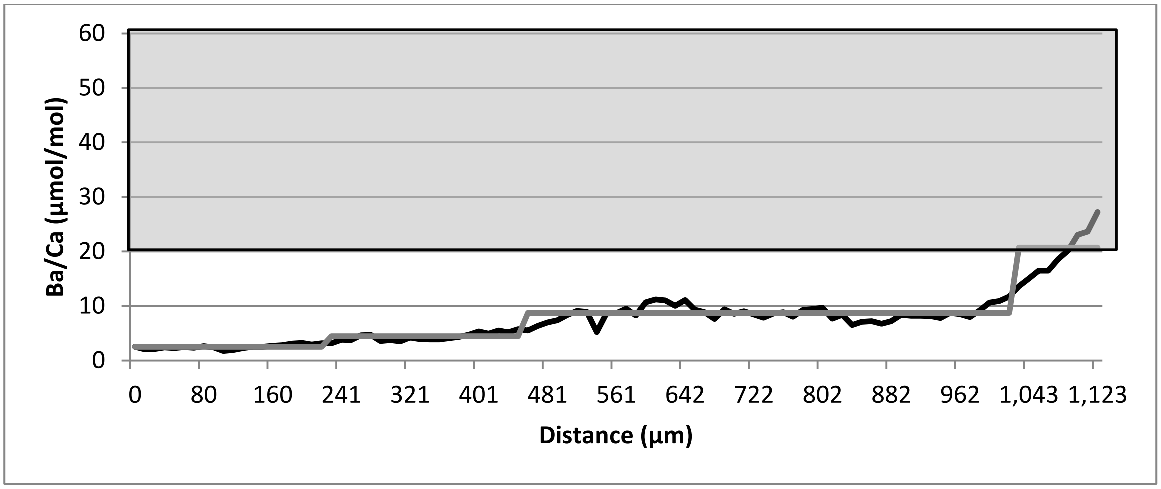
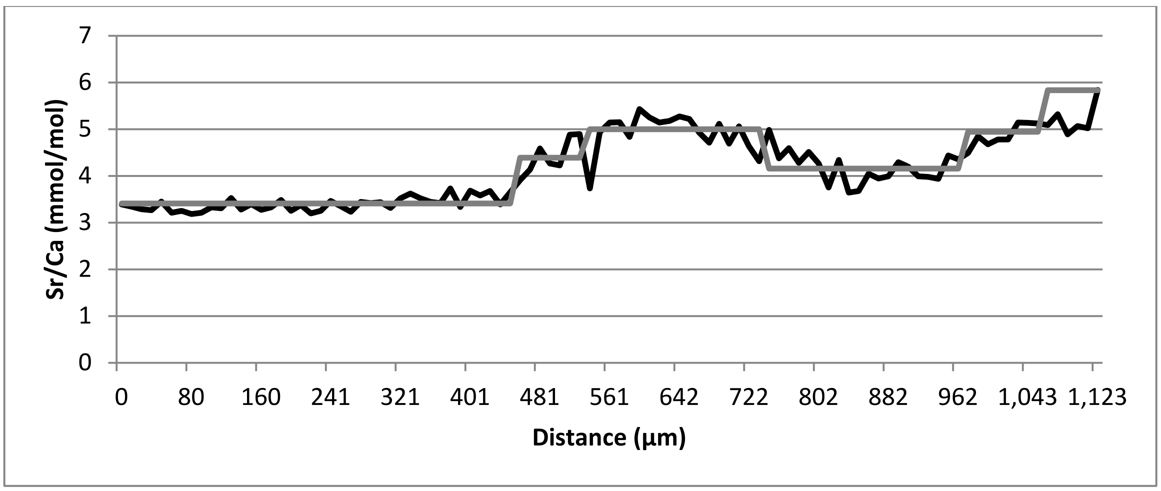


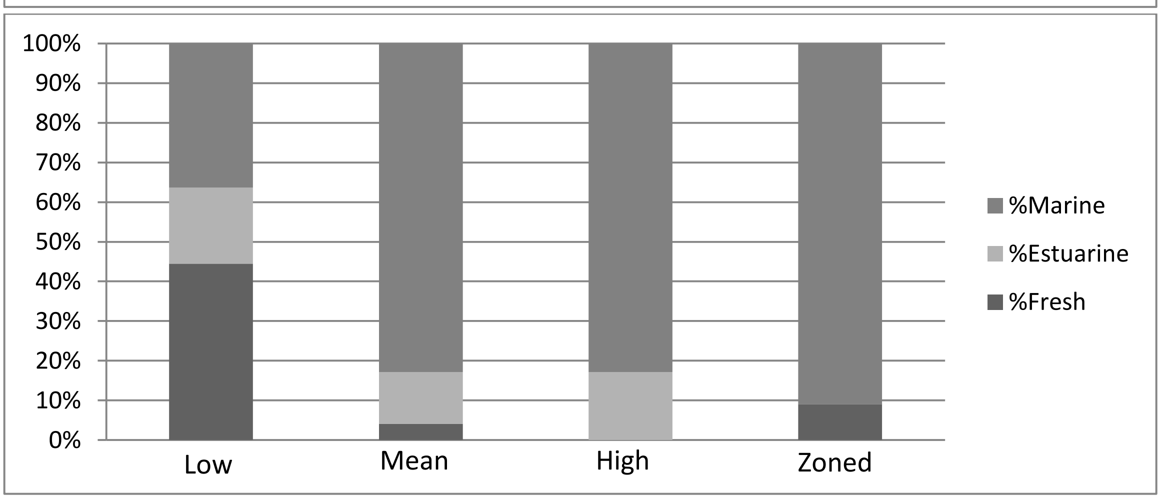
Figure AB.202. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 503.1. Figure 202.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

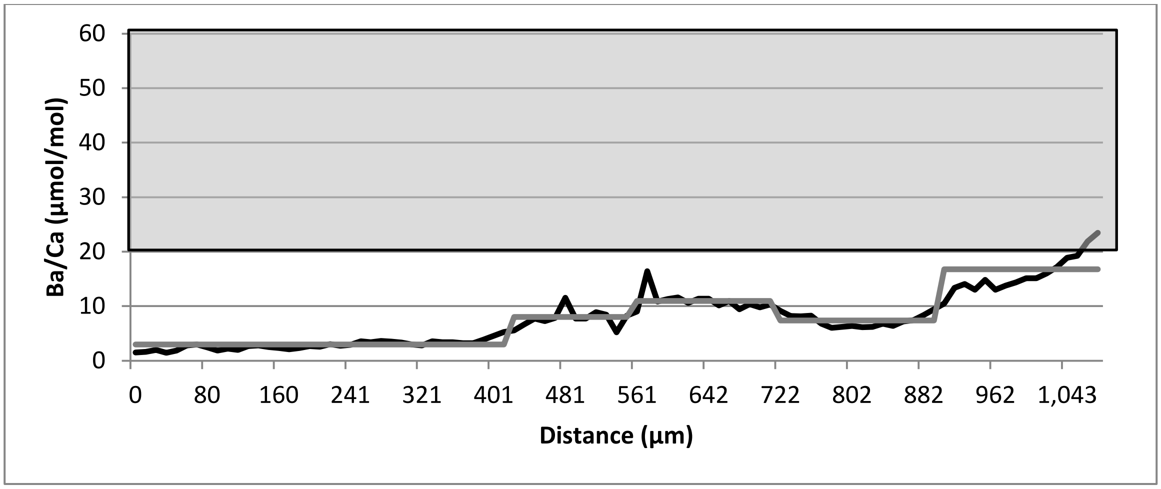


B.

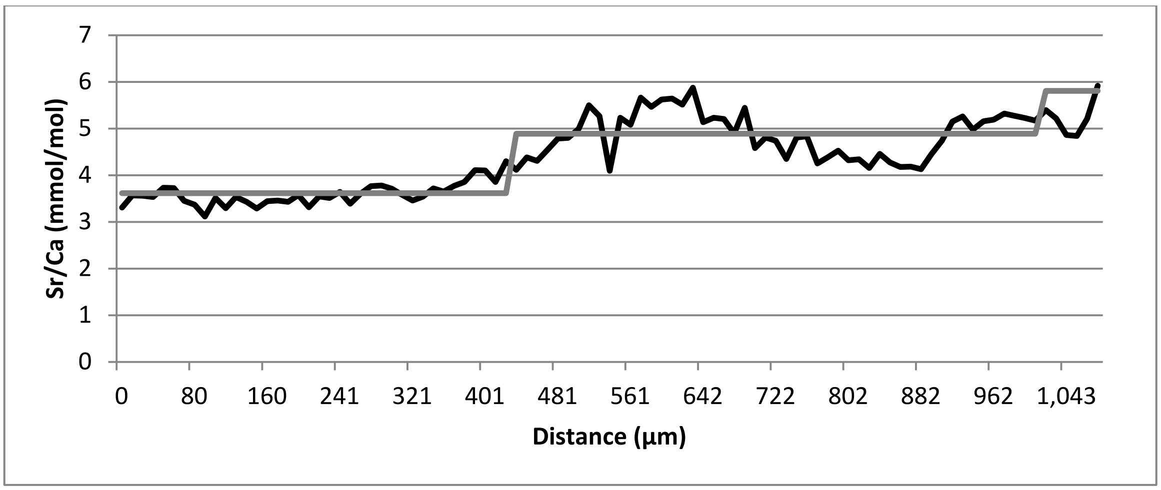


C.

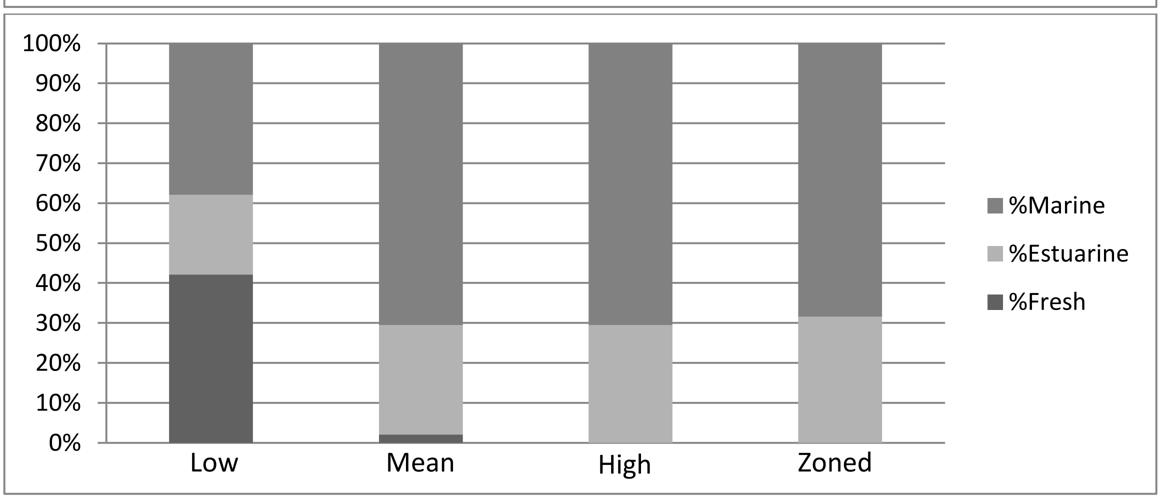
Figure AB.203. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 504. Figure 203.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

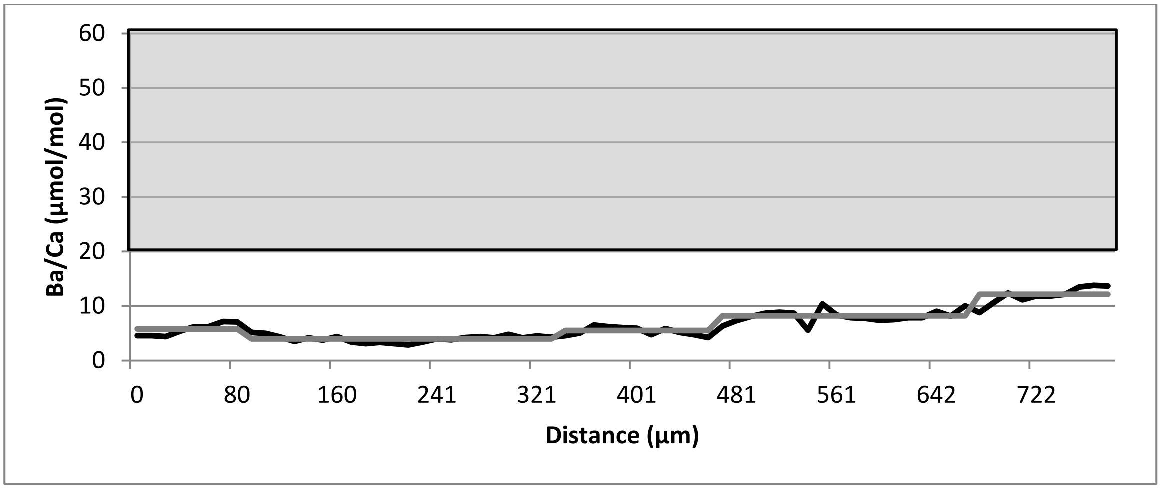


B.

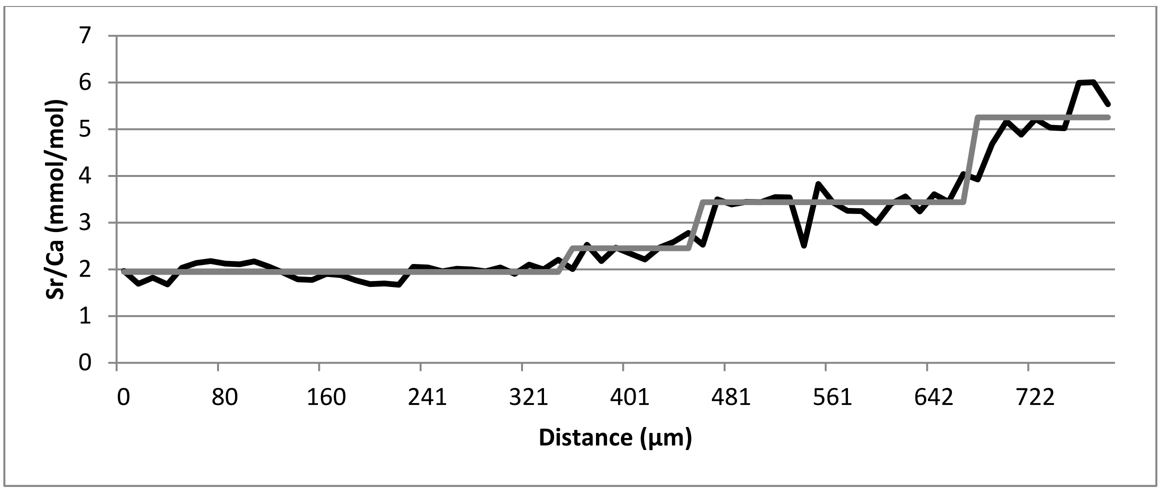


C.

Figure AB.204. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 507. Figure 204.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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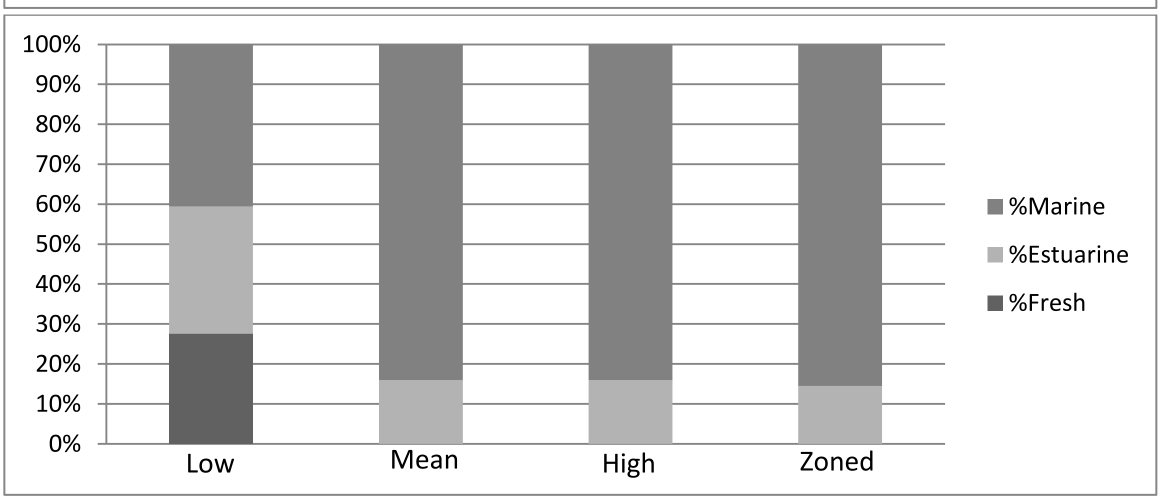
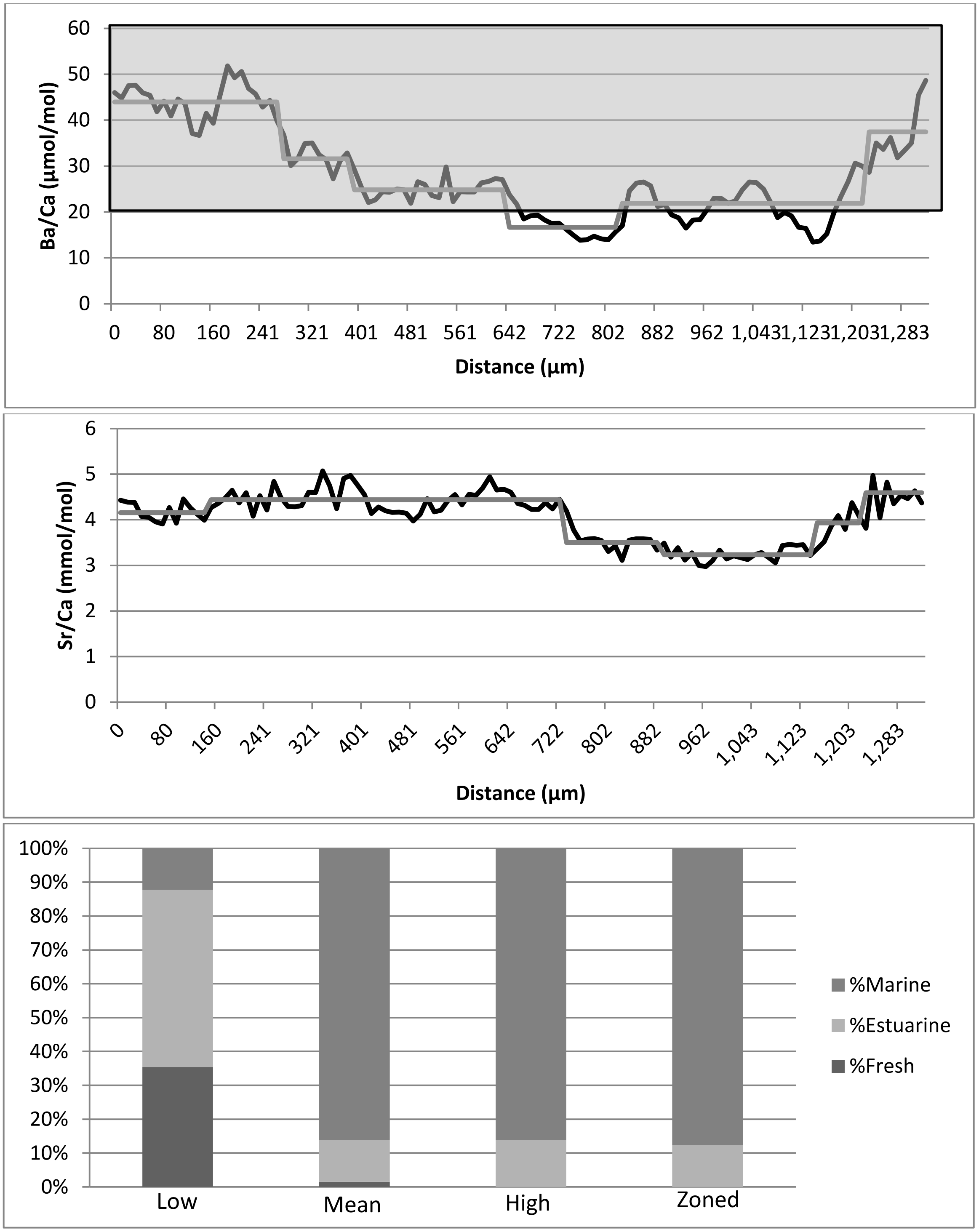


C.

Figure AB.205. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 509. Figure 205.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

B.

C.

Figure AB.206. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 513. Figure 206.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

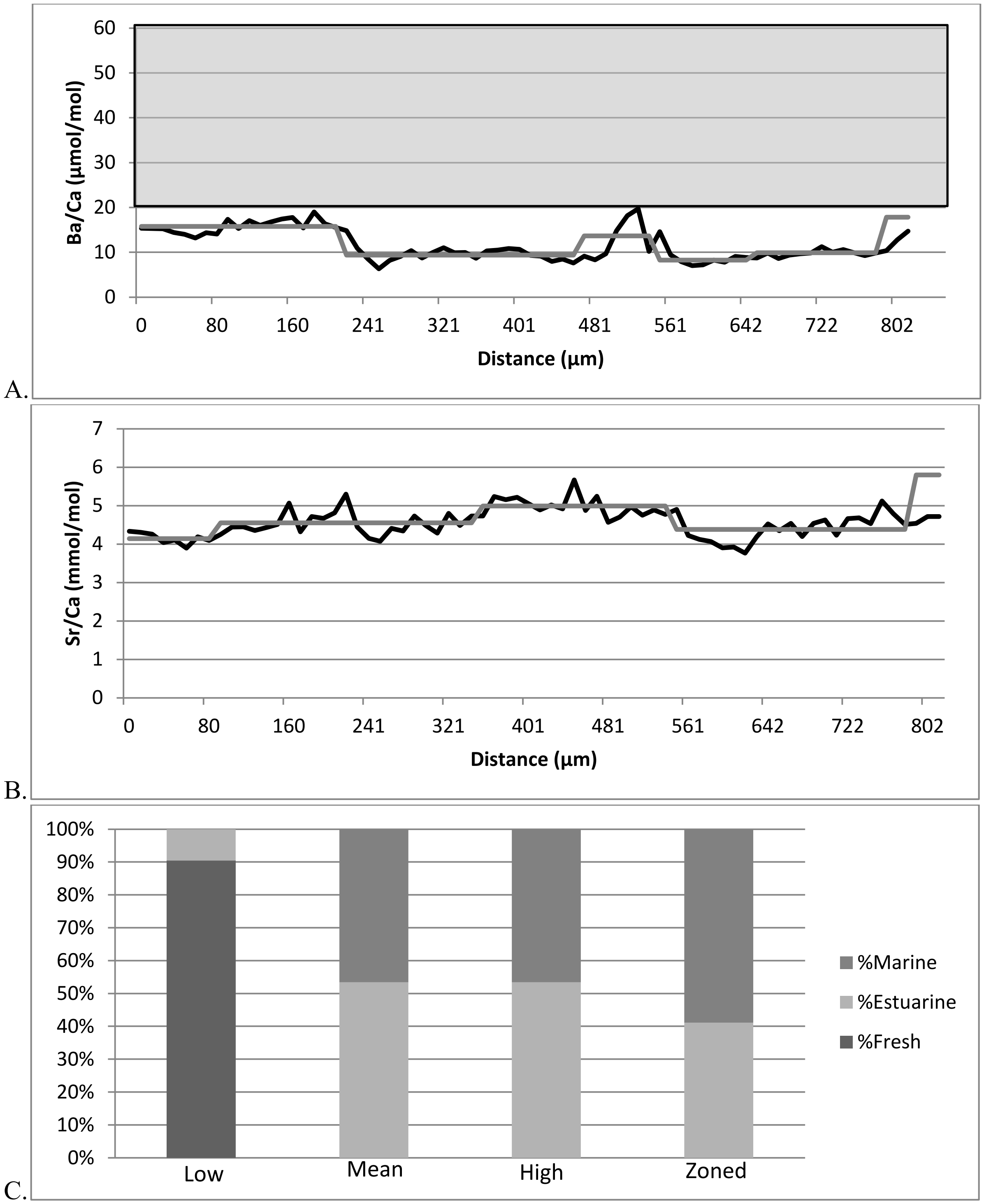
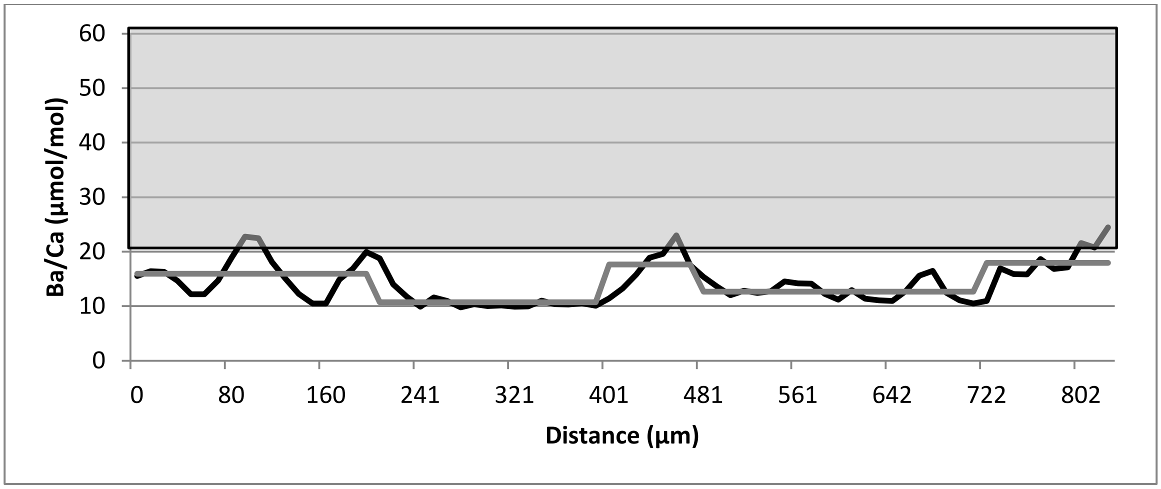
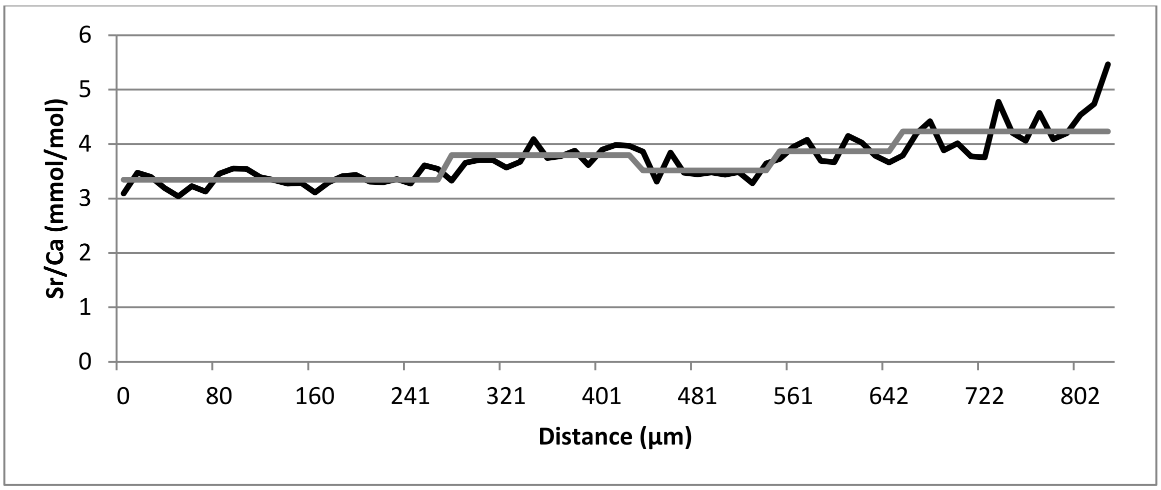


Figure AB.207. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 514. Figure 207.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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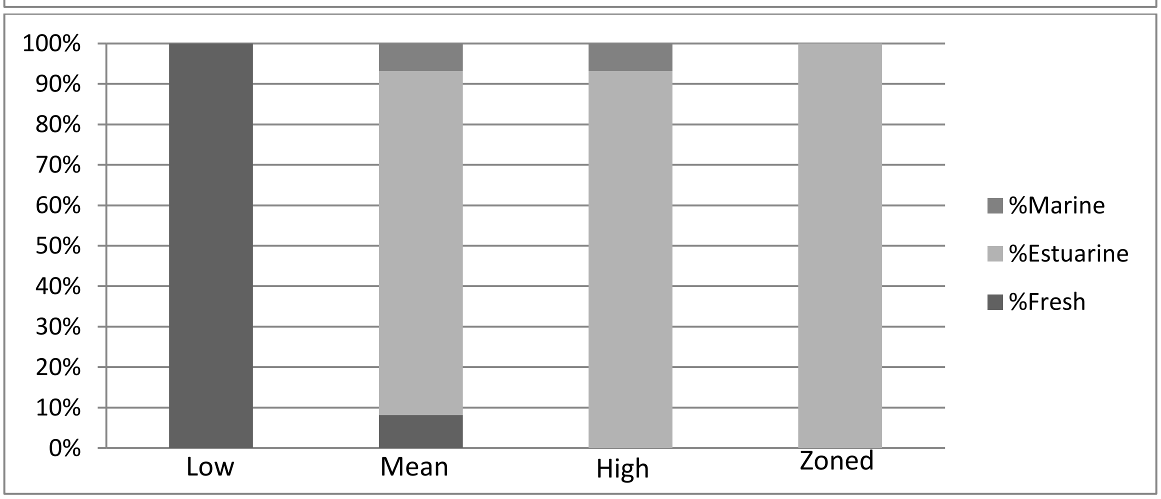
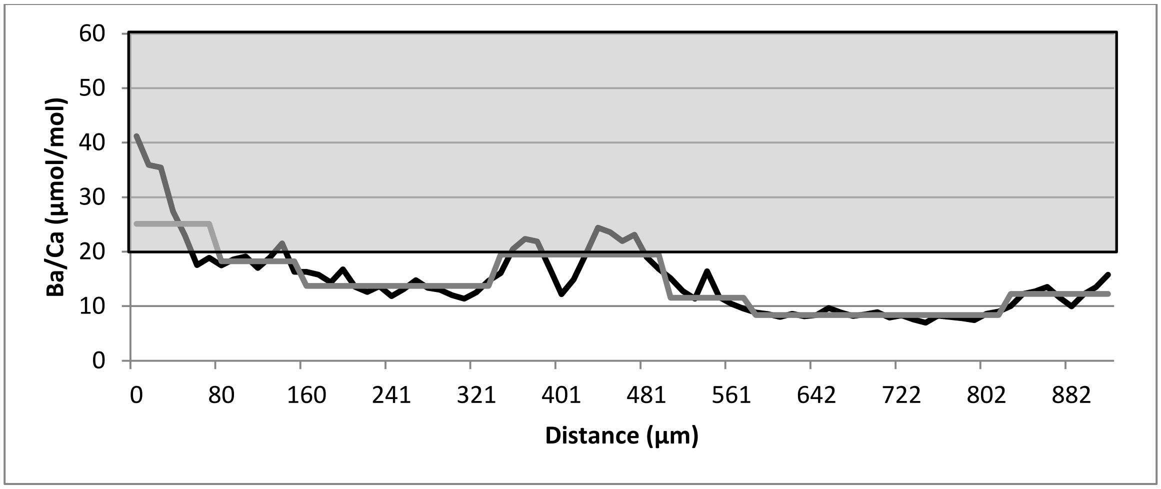
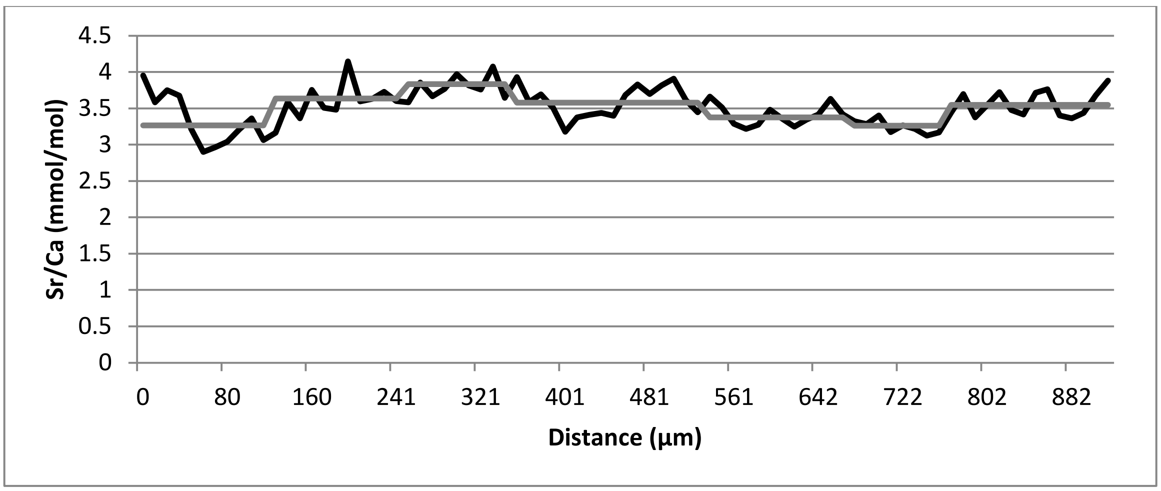


C.

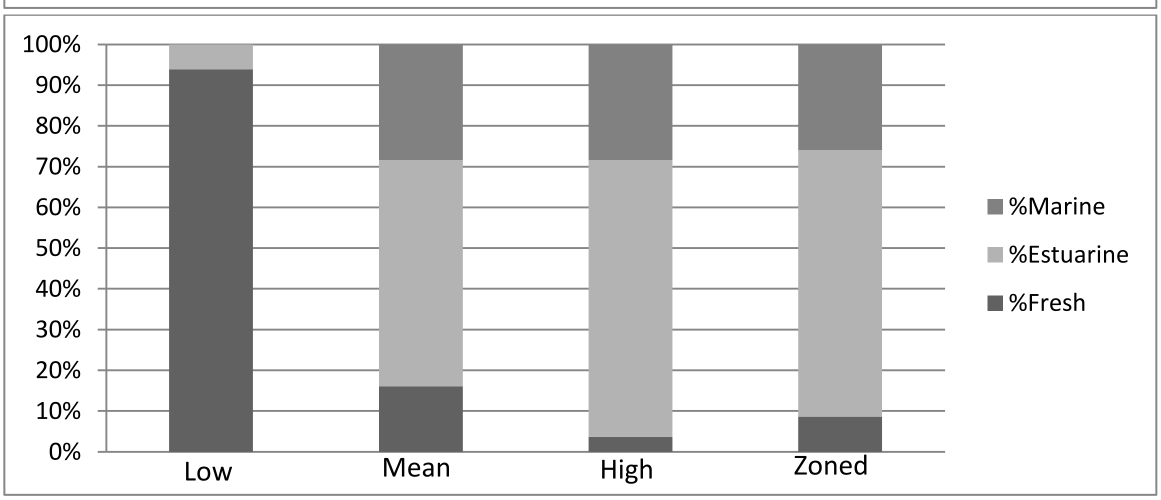
Figure AB.208. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 516. Figure 208.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.209. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 518. Figure 209.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

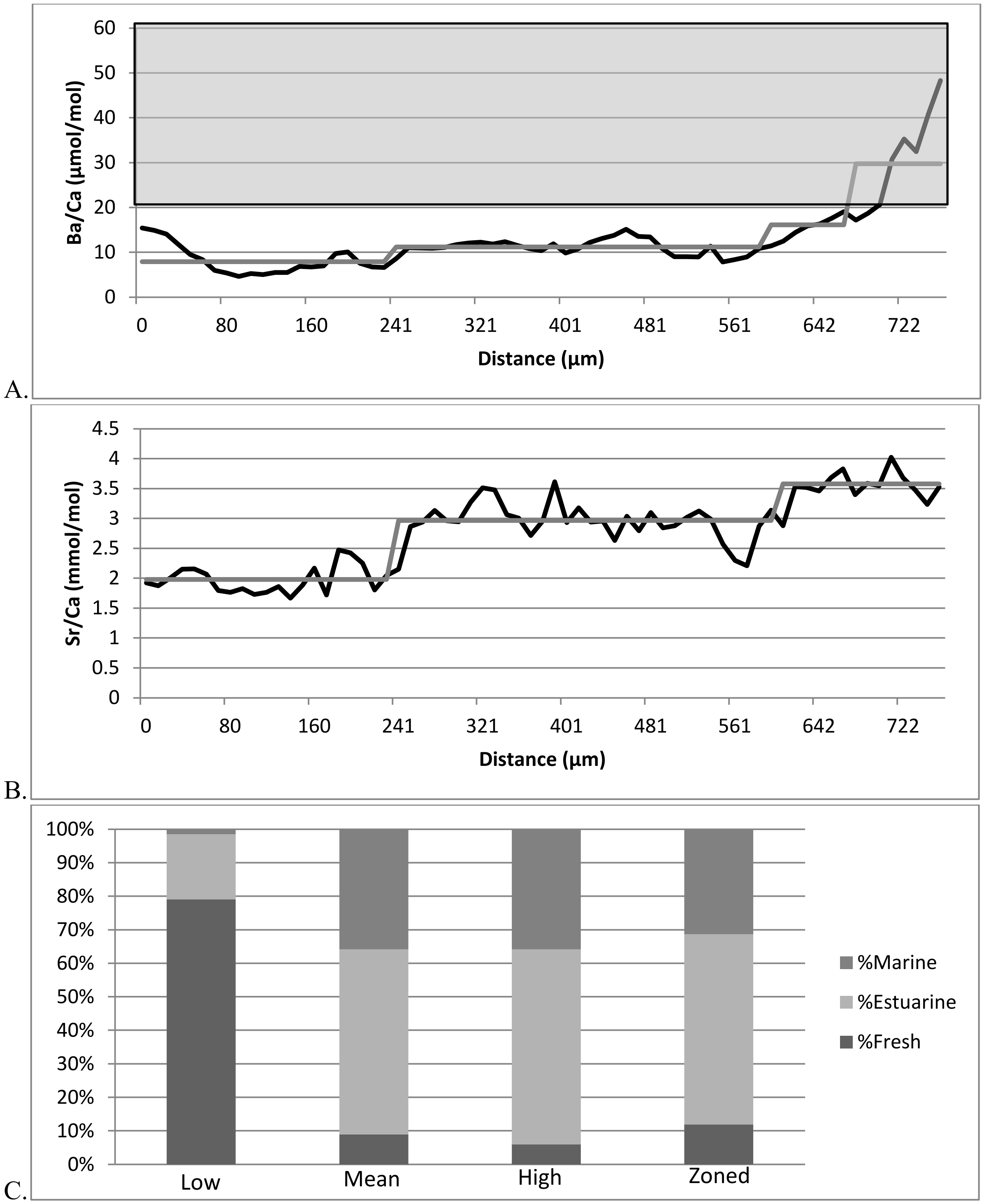
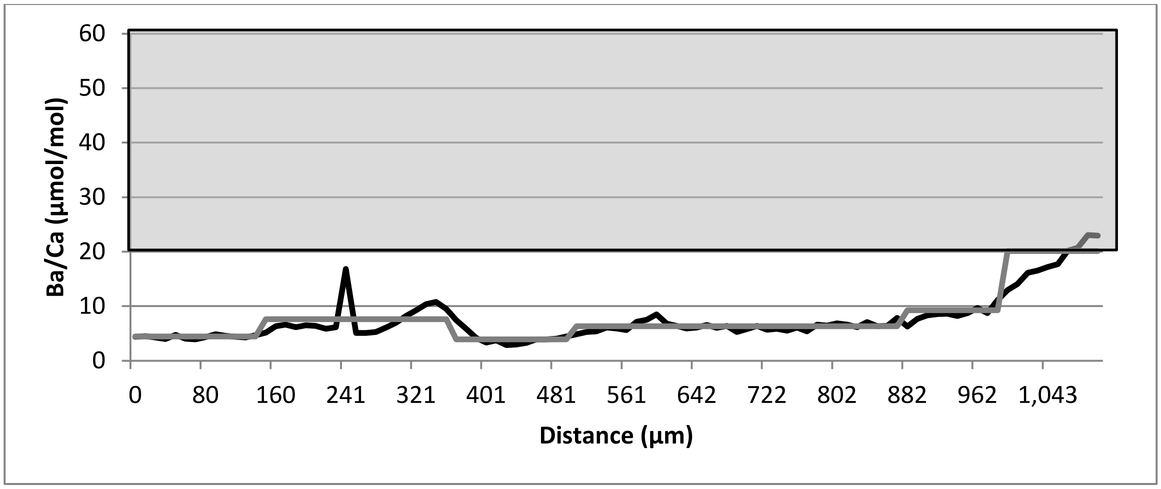
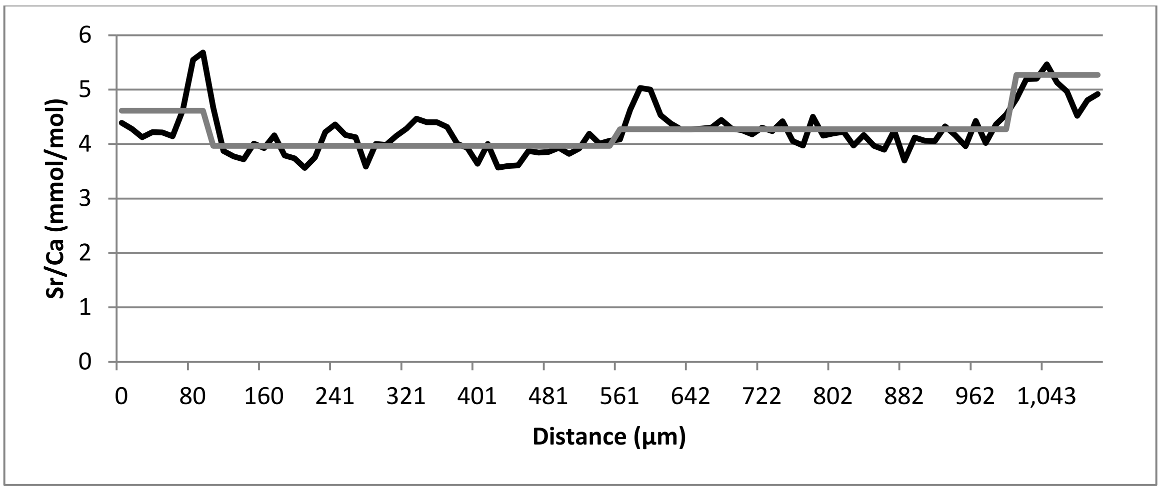


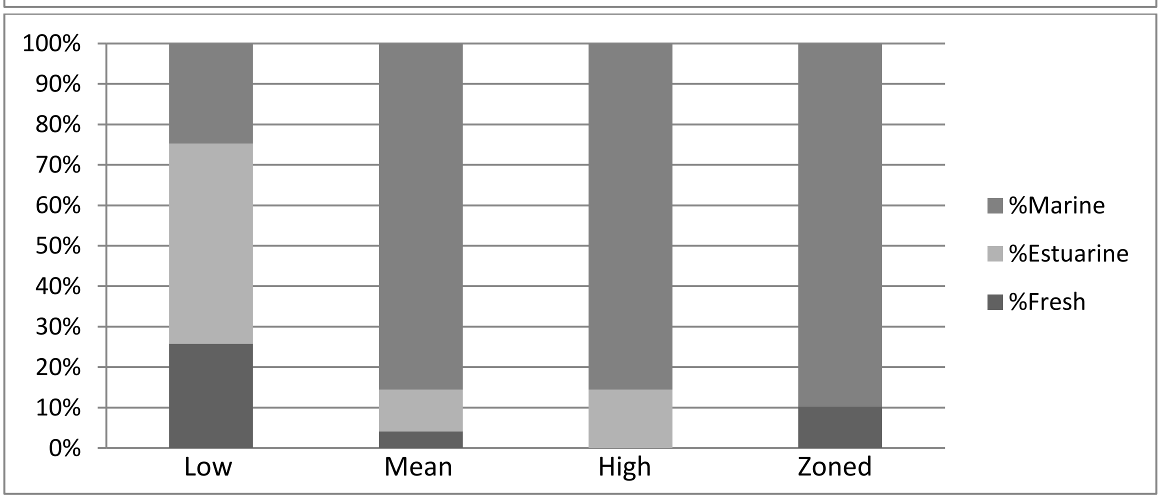
Figure AB.210. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 519. Figure 210.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

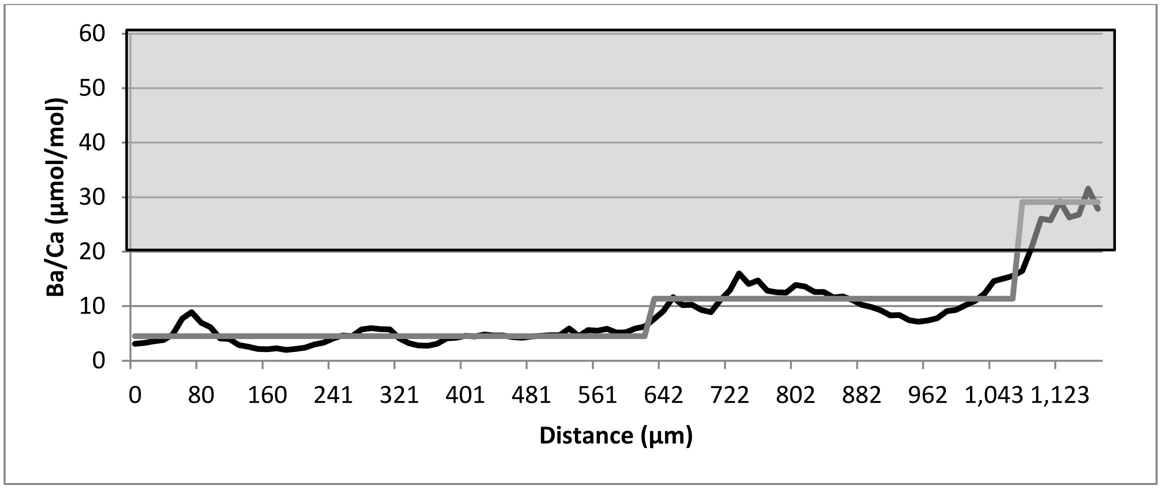


B.

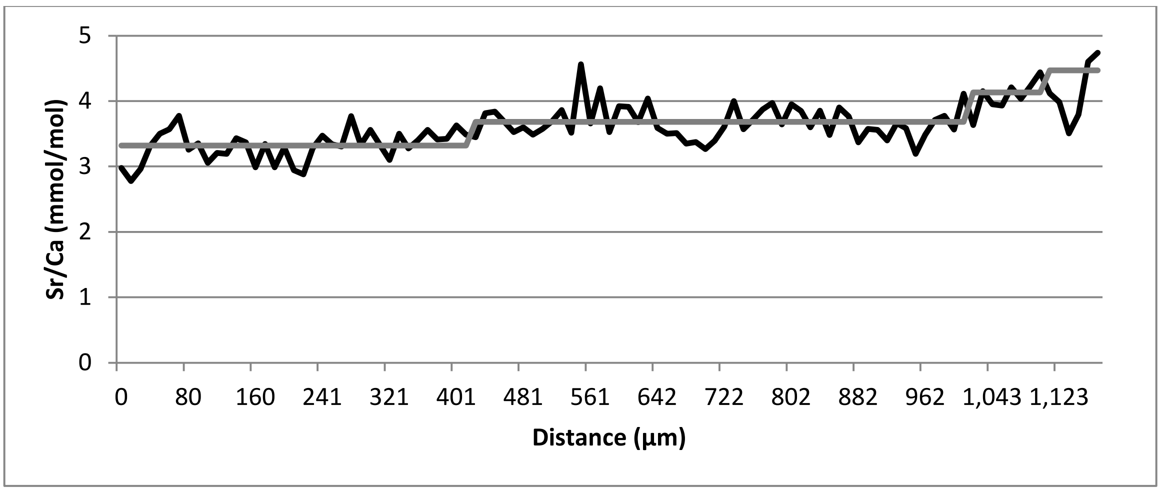


C.

Figure AB.211. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 520. Figure 211.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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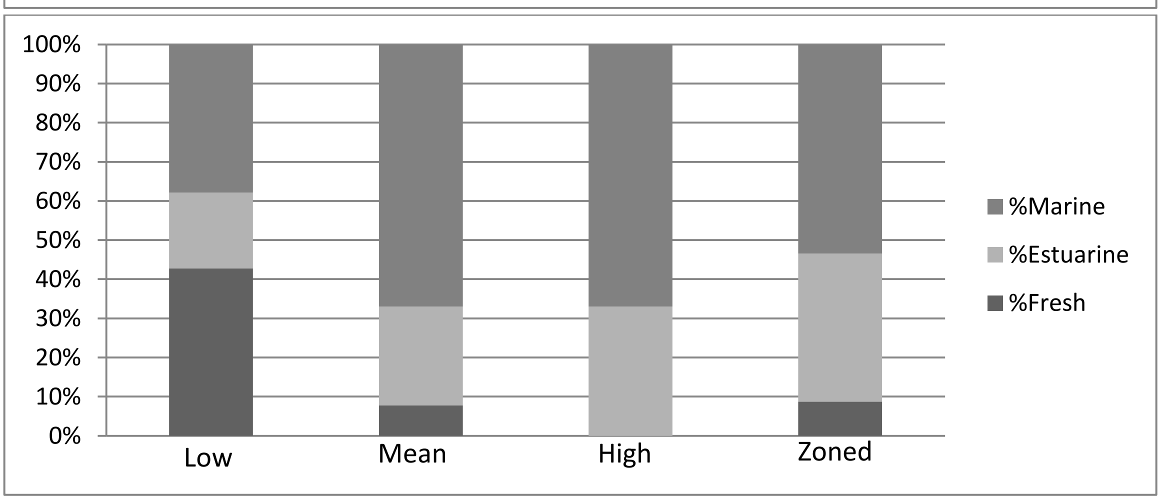
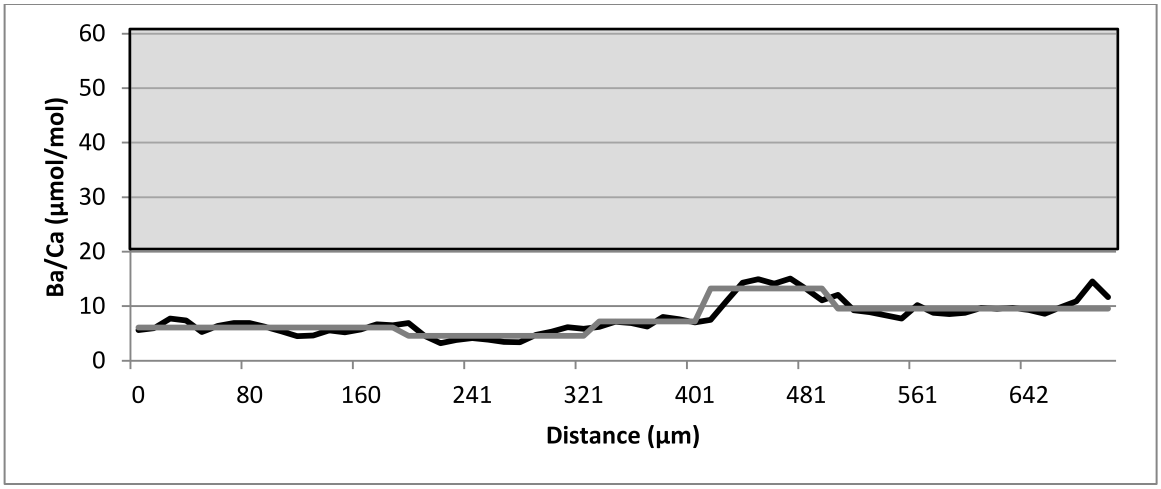
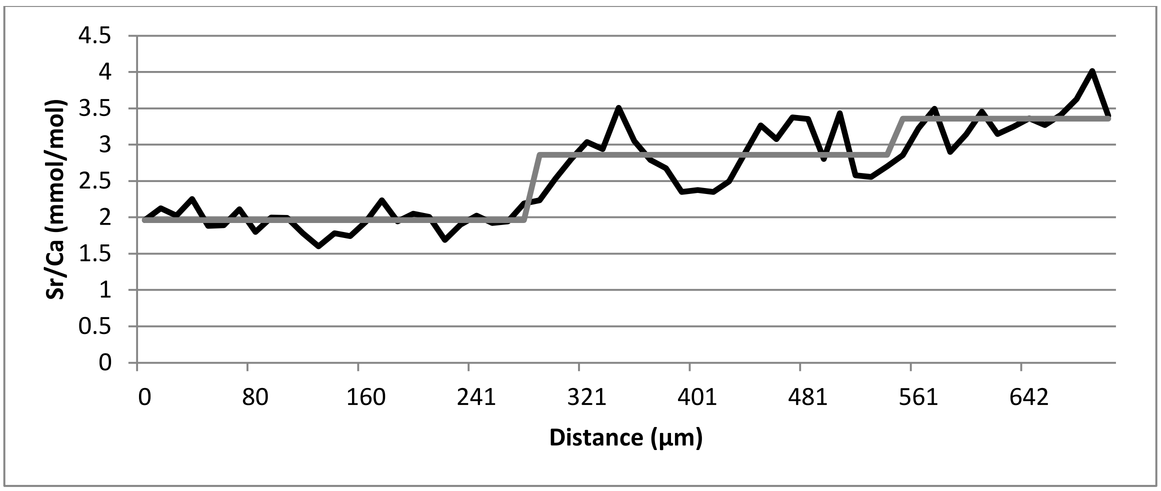


C.

Figure AB.212. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 522. Figure 212.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

A.


B.


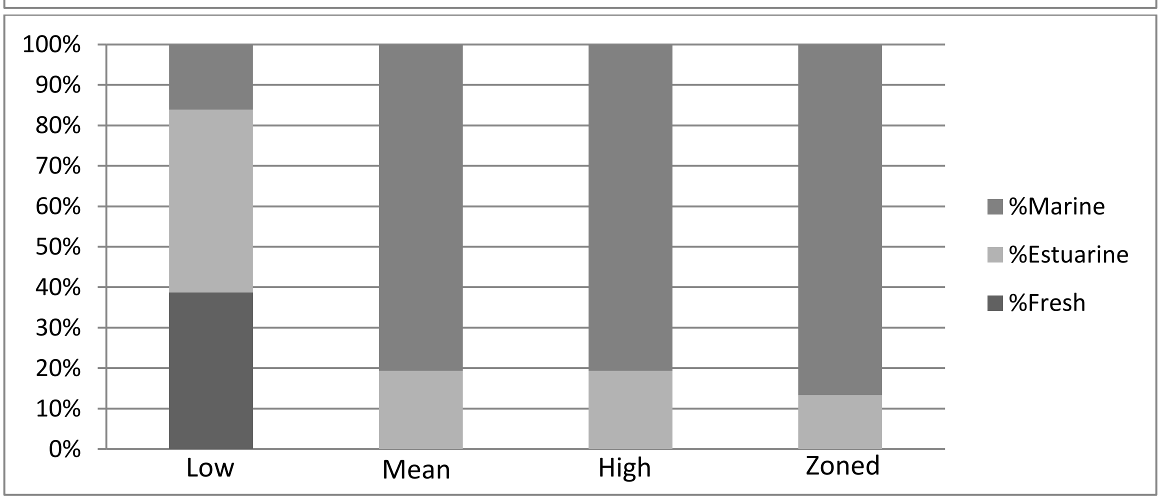
C.


Figure AB.213. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 415. Figure 213.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

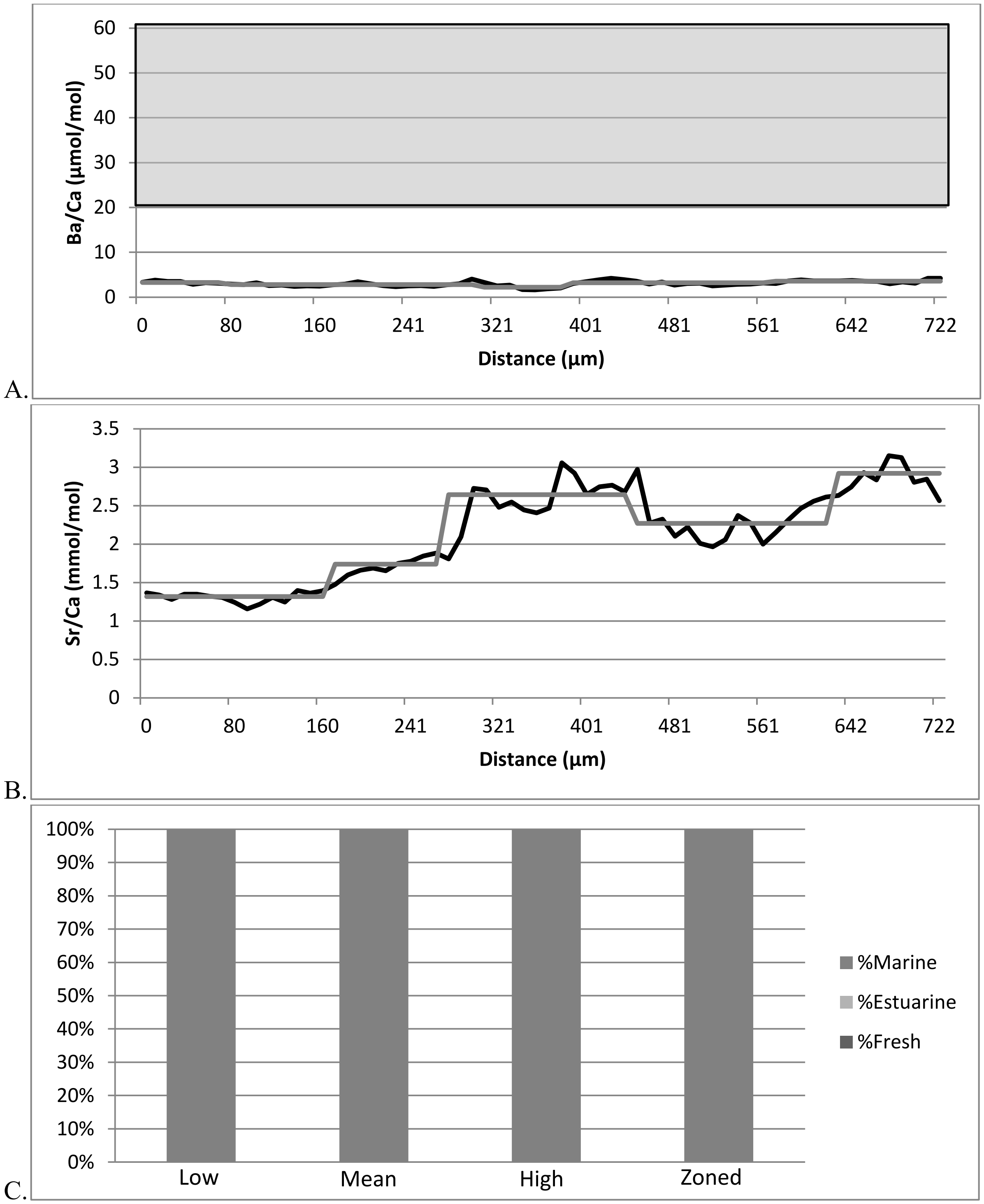


Figure AB.214. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 440. Figure 214.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

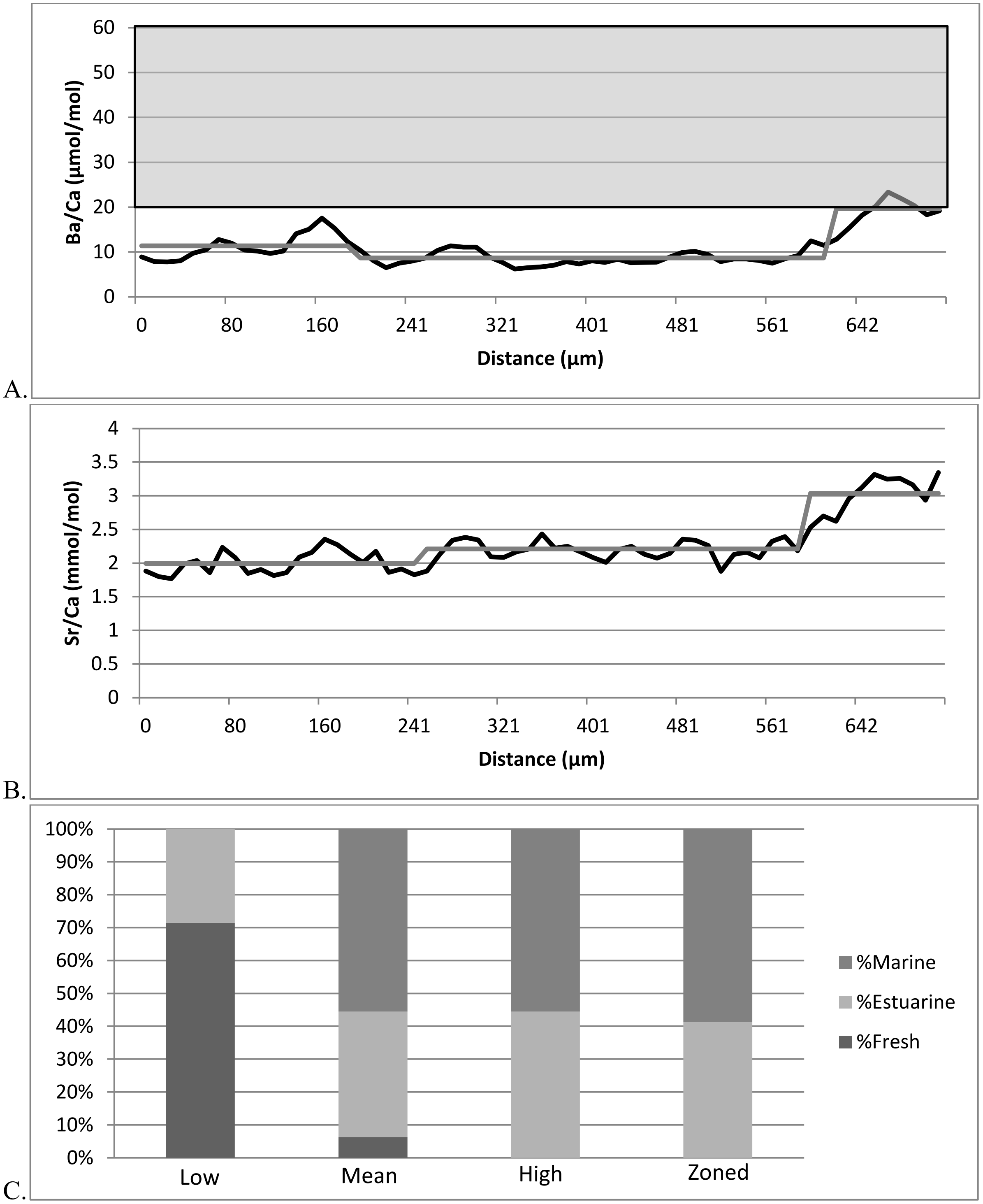
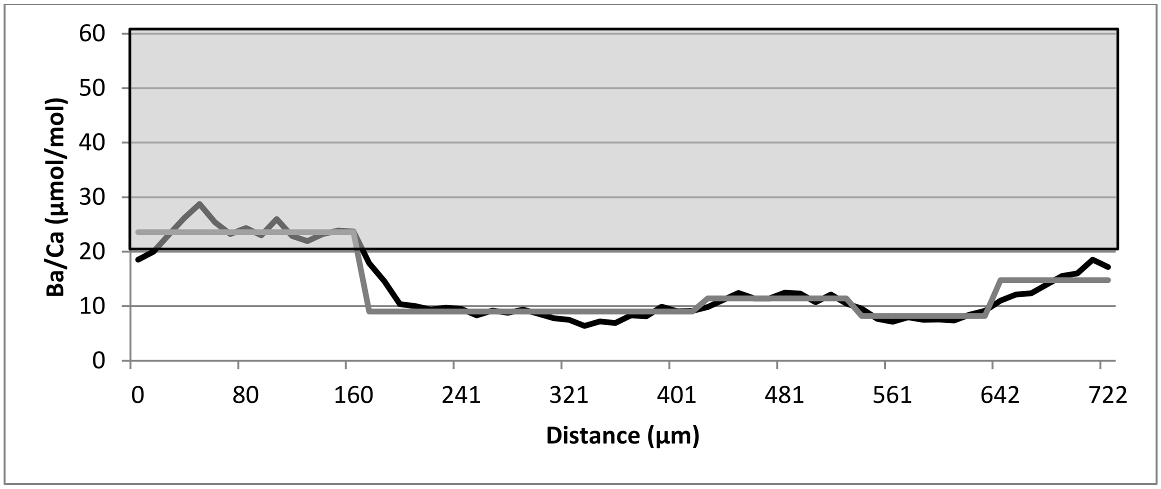
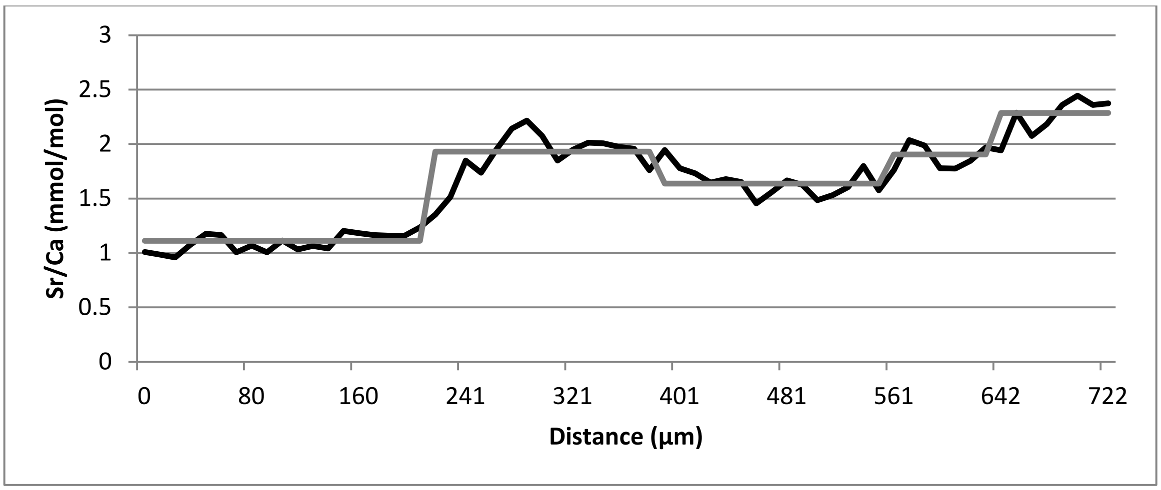


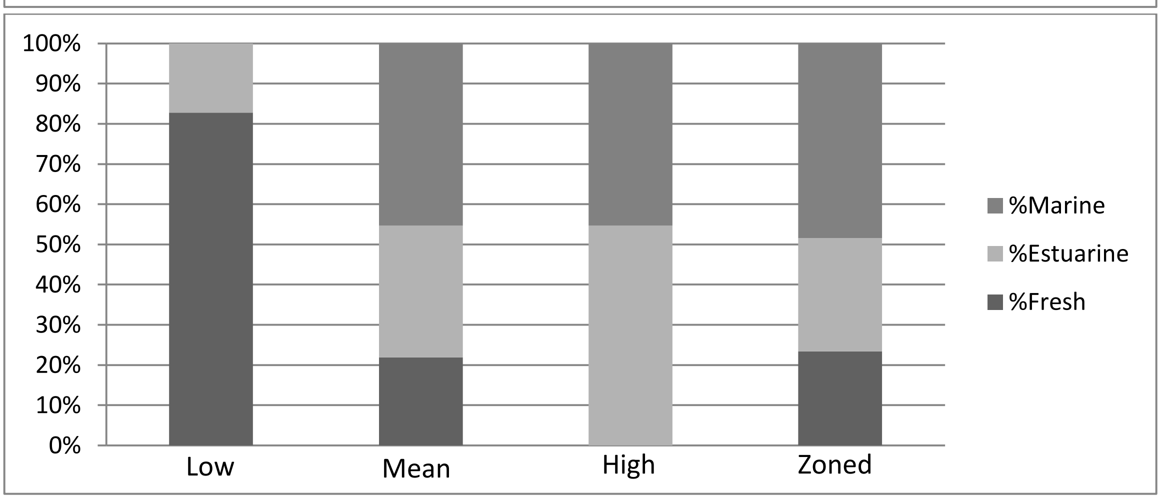
Figure AB.215. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 445. Figure 215.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

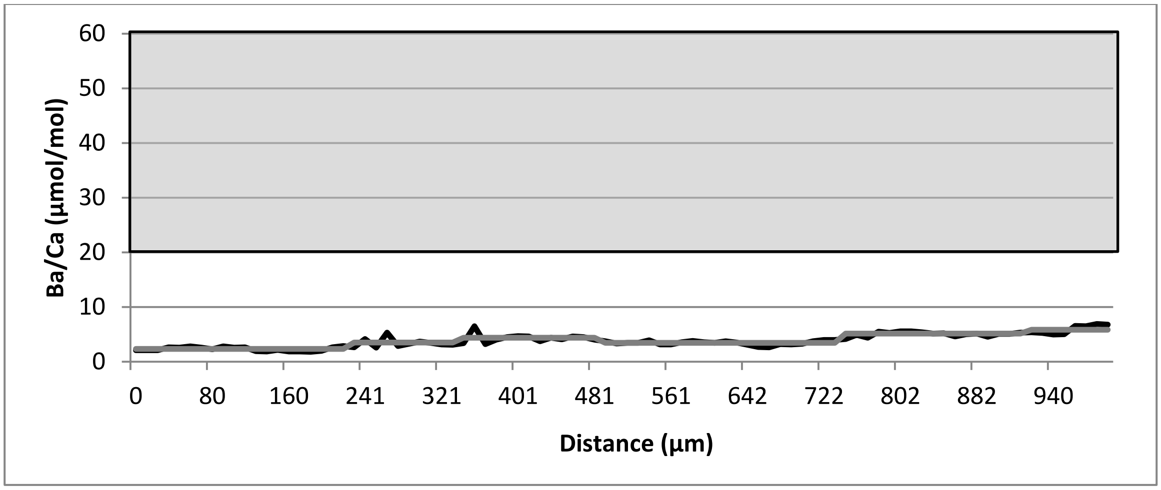


B.

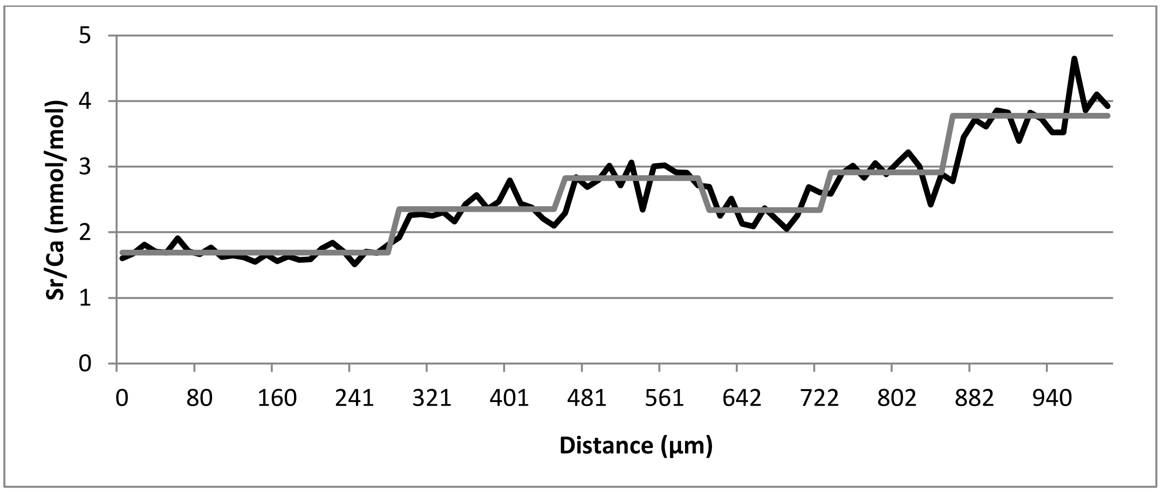


C.

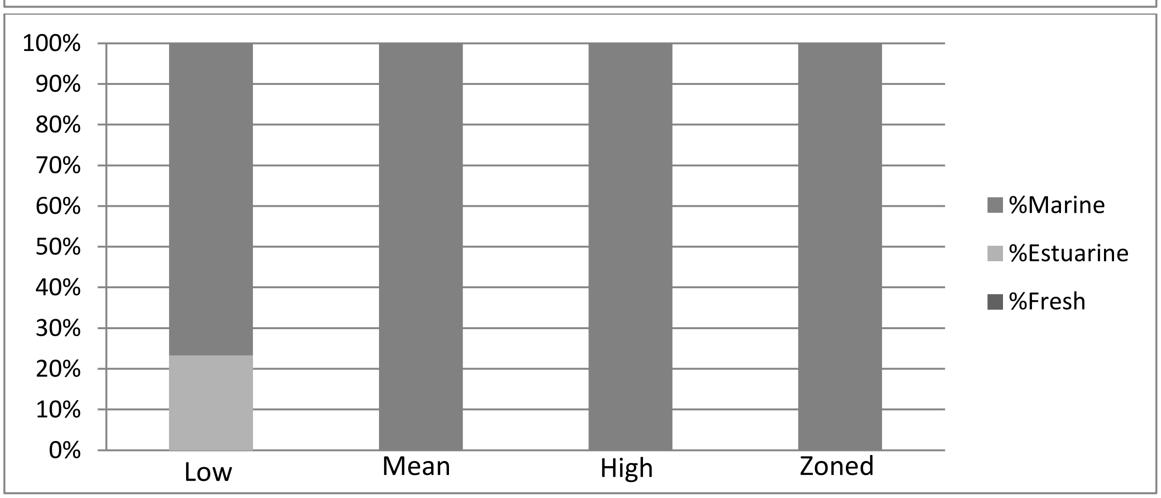
Figure AB.216. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 447. Figure 216.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

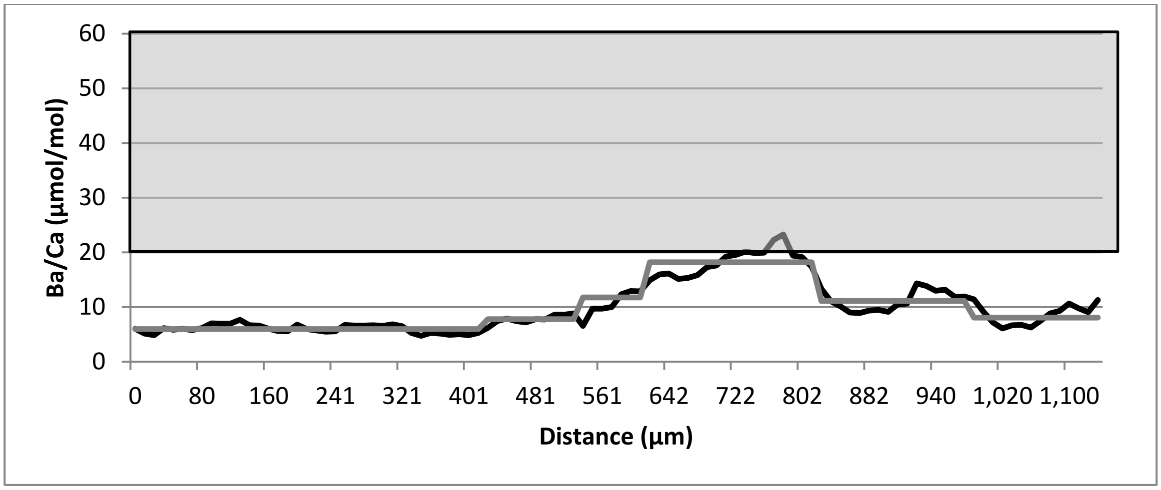


B.

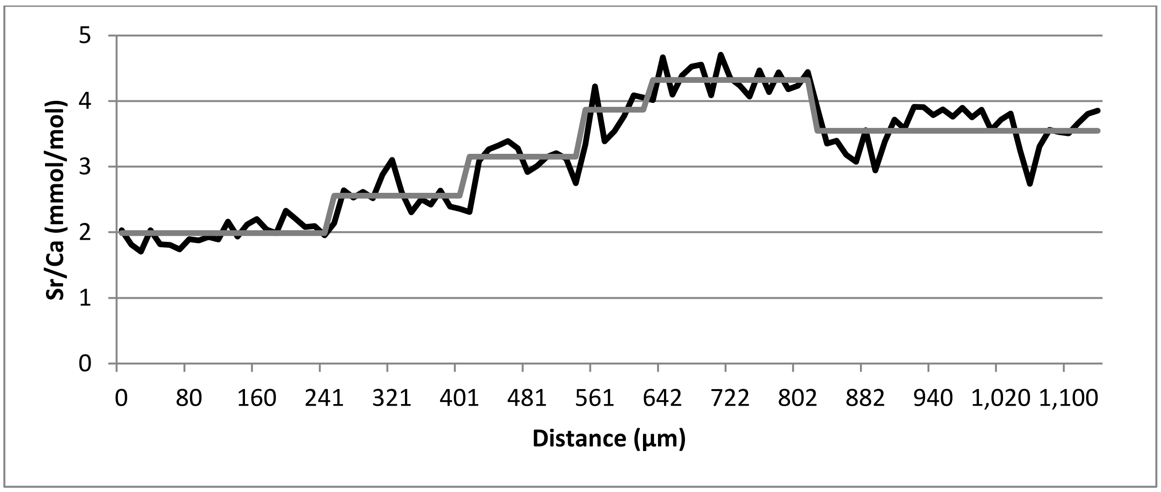


C.

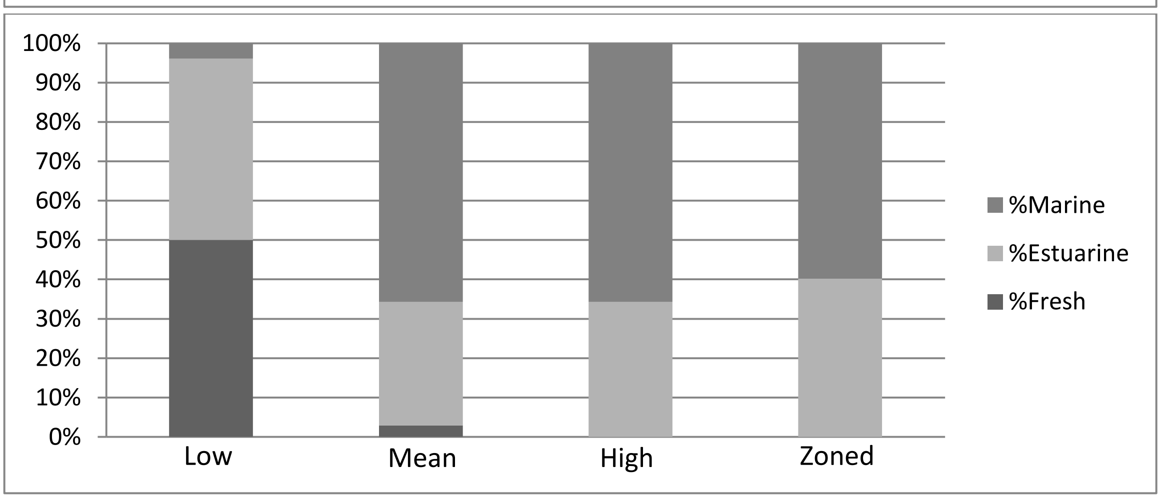
Figure AB.217. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 453. Figure 217.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.

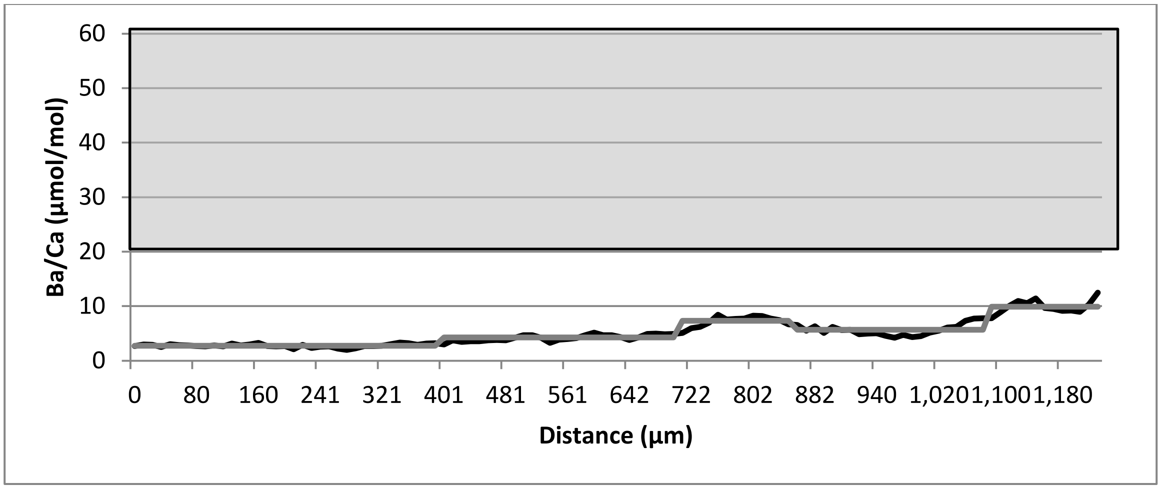


B.

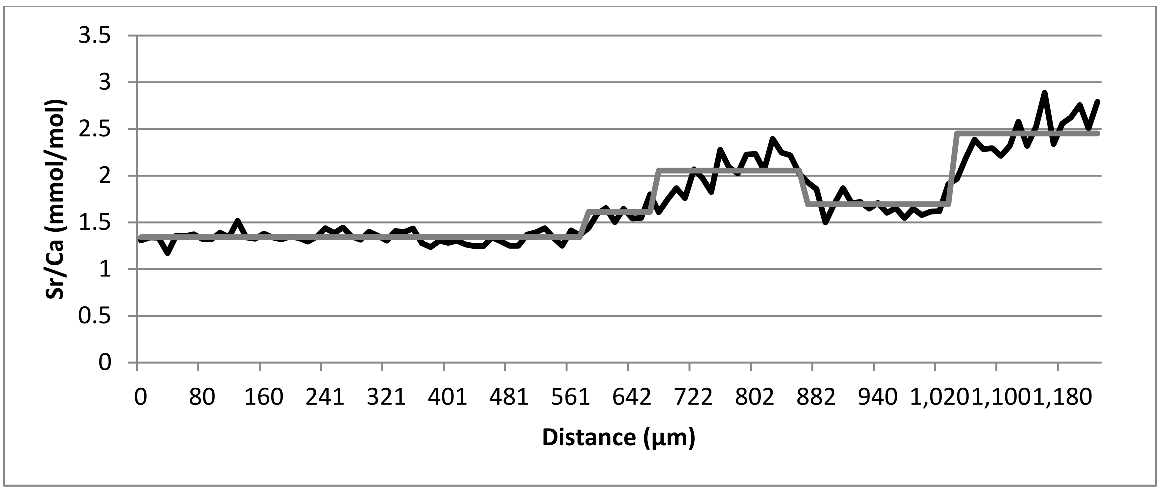


C.

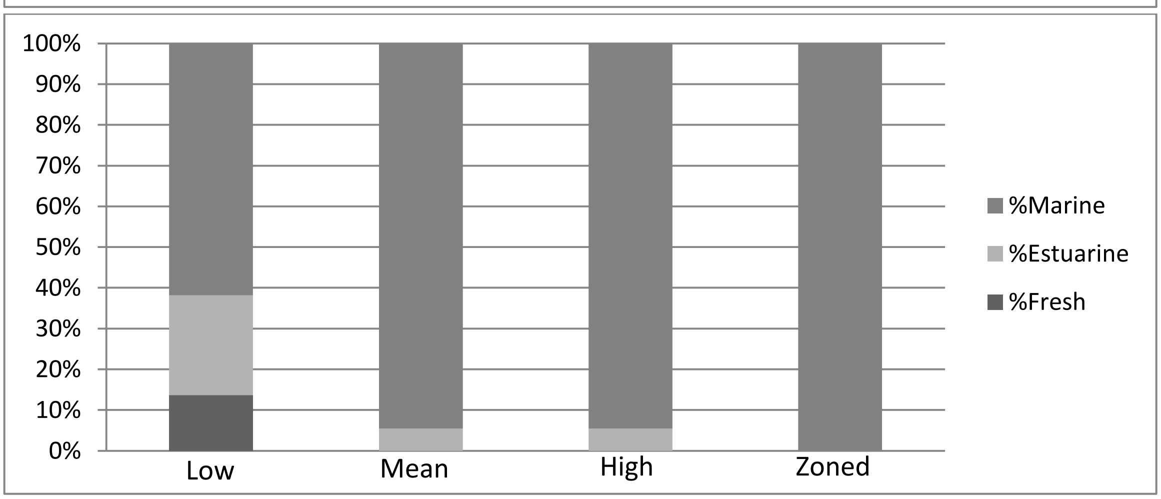
Figure AB.218. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 501. Figure 218.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



B.



C.

Figure AB.219. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 511. Figure 219.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

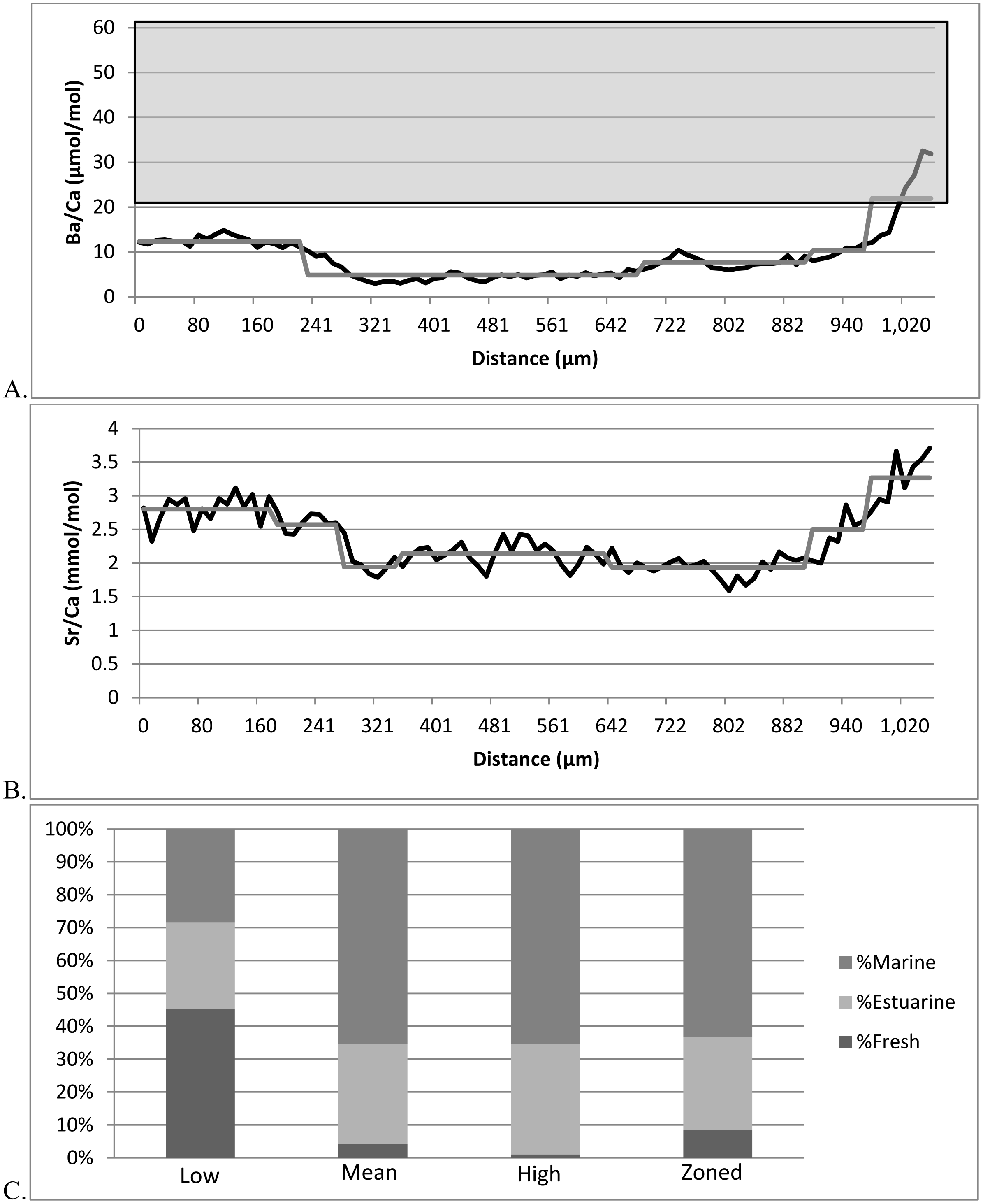
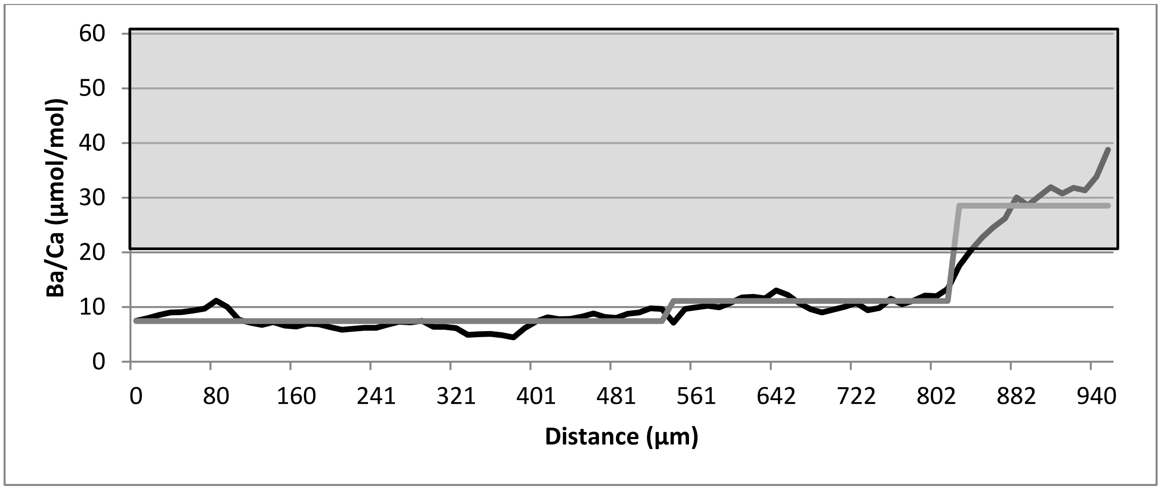
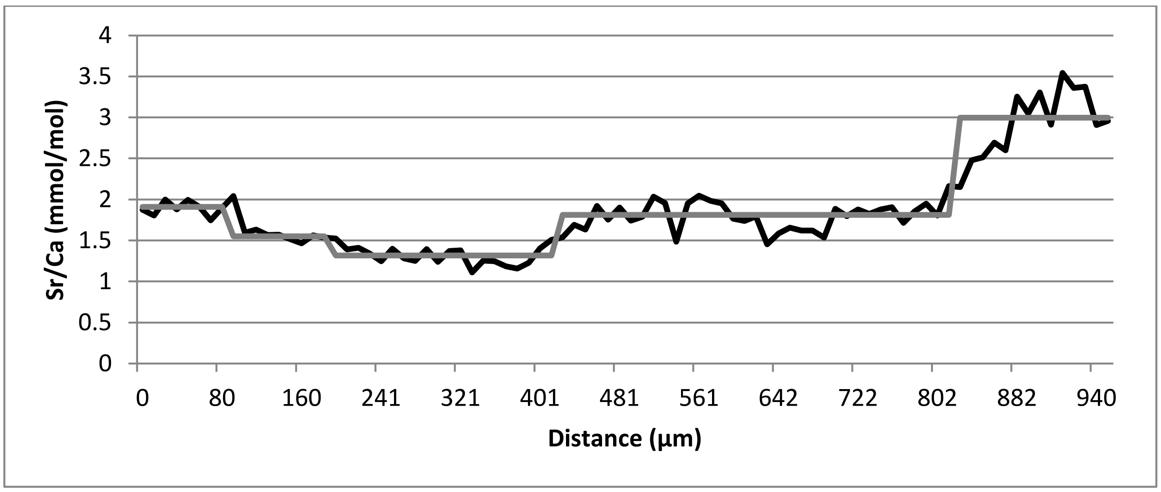


Figure AB.220. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 525. Figure 220.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.


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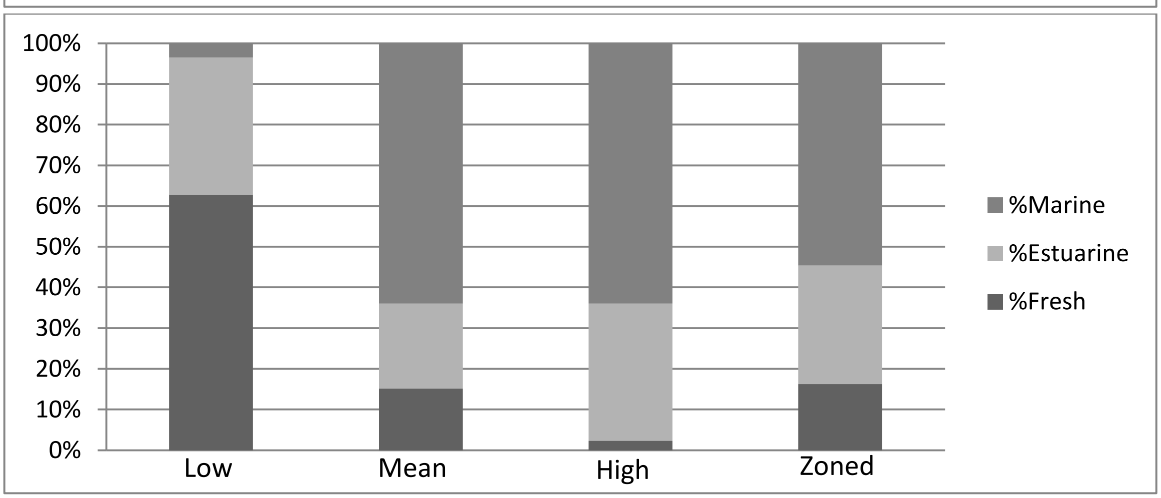


C.

Figure AB.221. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 529. Figure 221.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

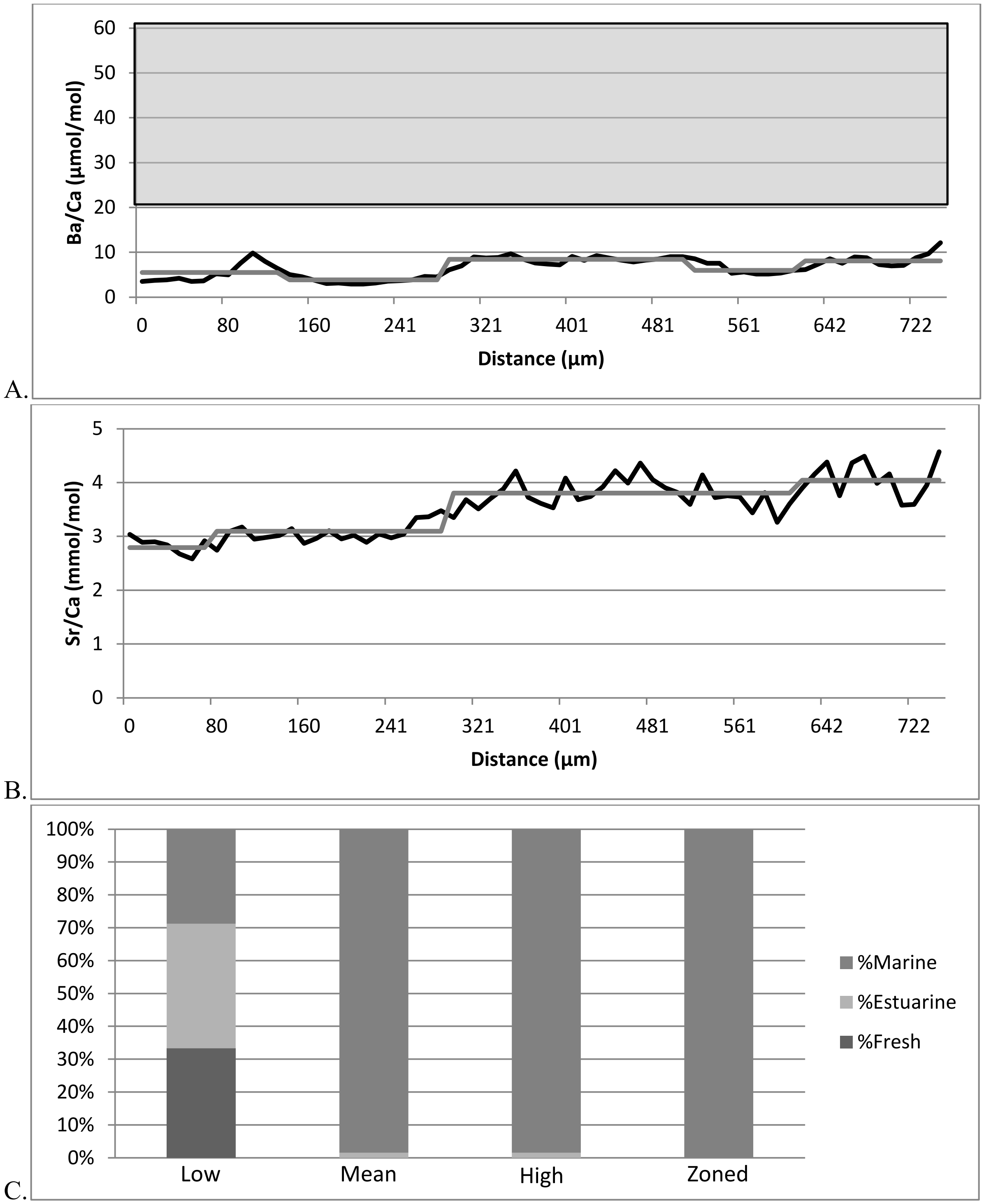
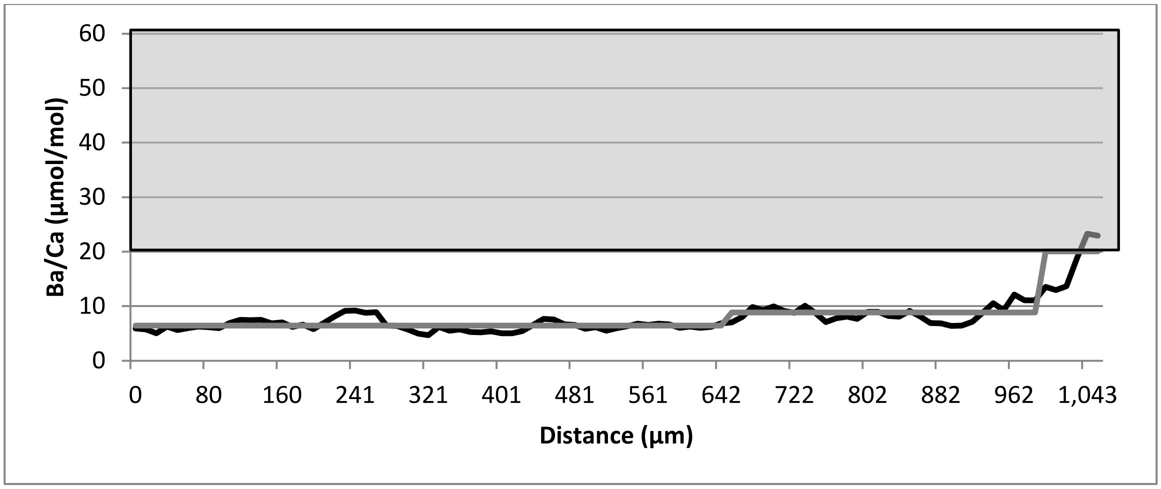
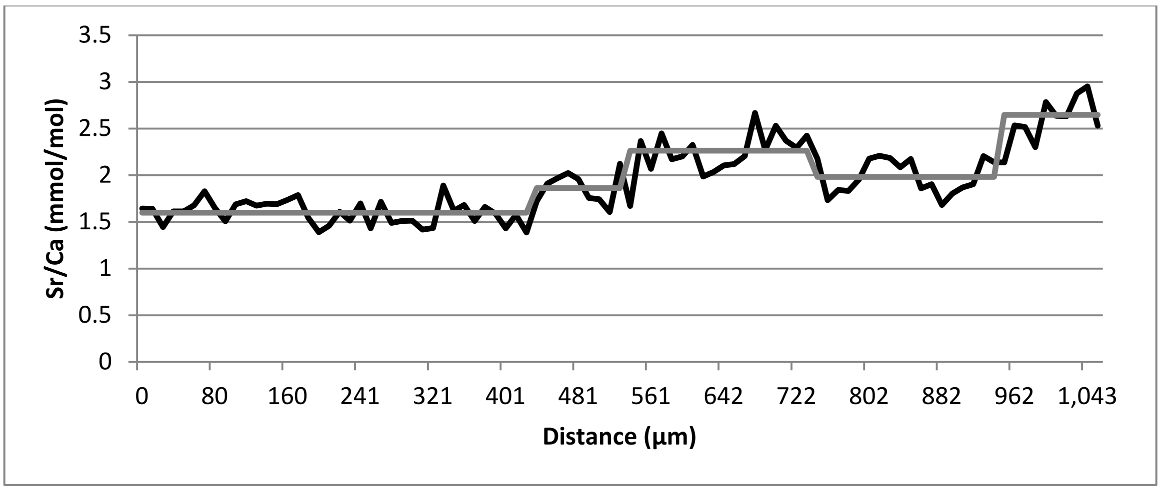


Figure AB.222. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 530. Figure 222.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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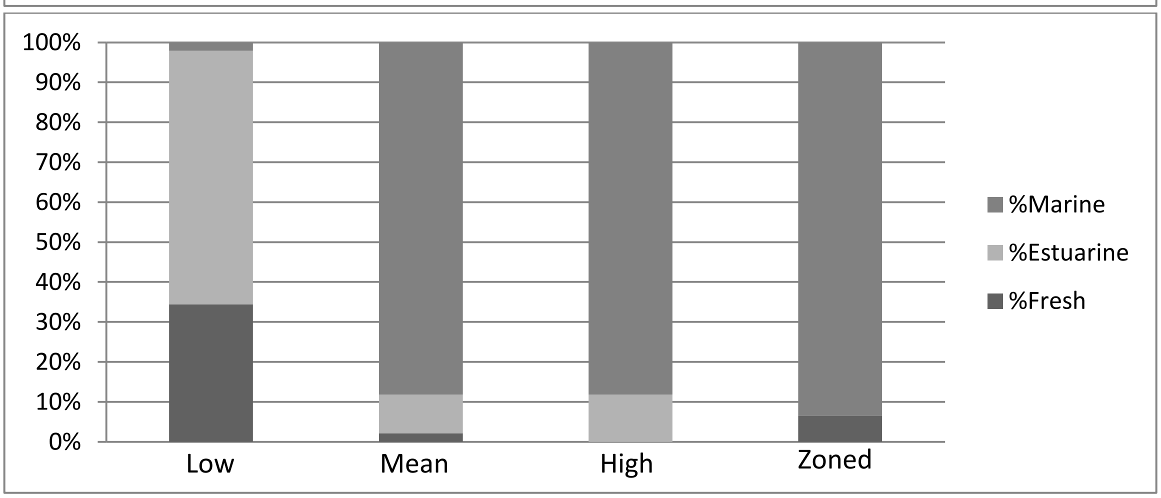
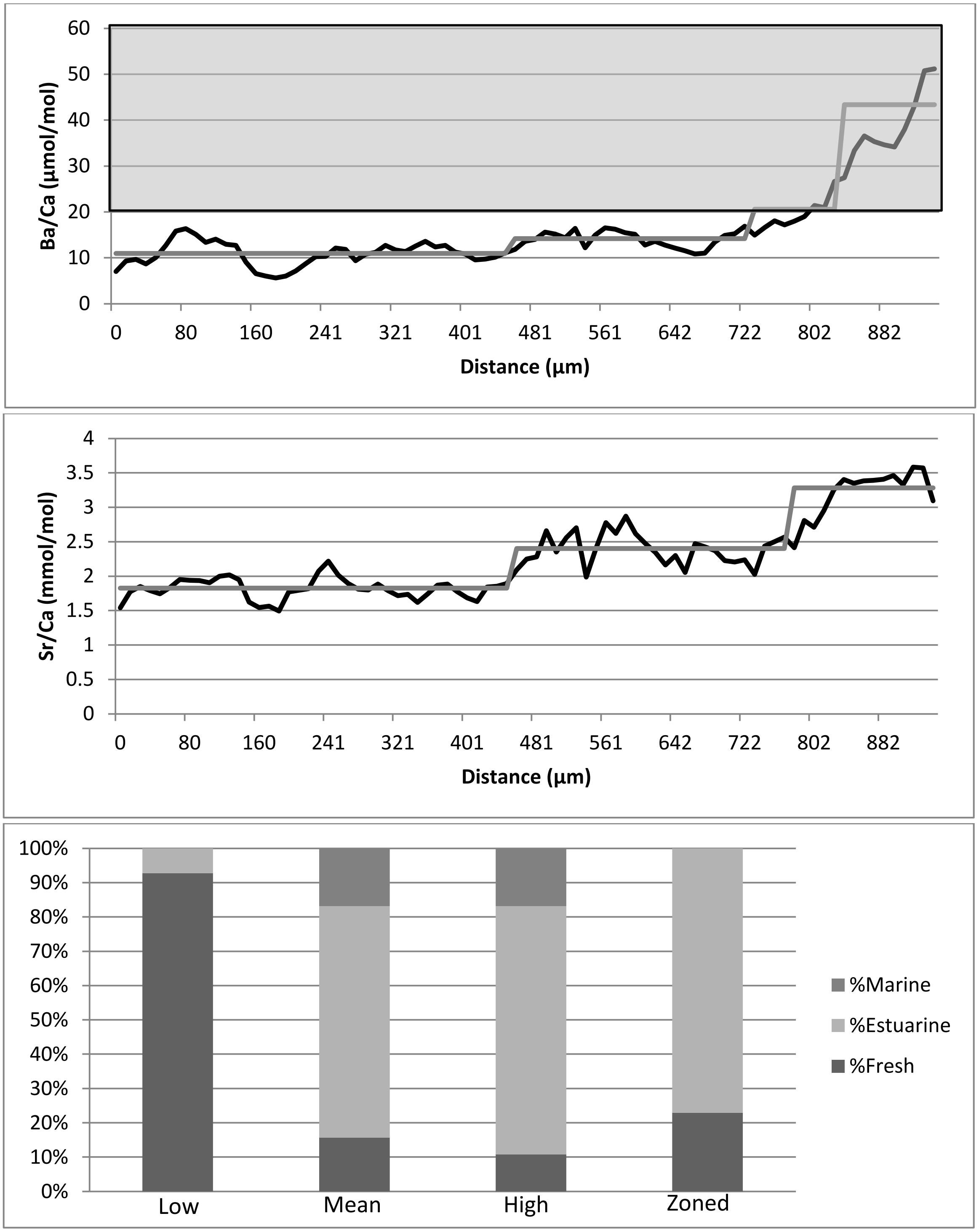


C.

Figure AB.223. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 531. Figure 223.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.
B.
C.

Figure AB.224. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 533. Figure 224.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

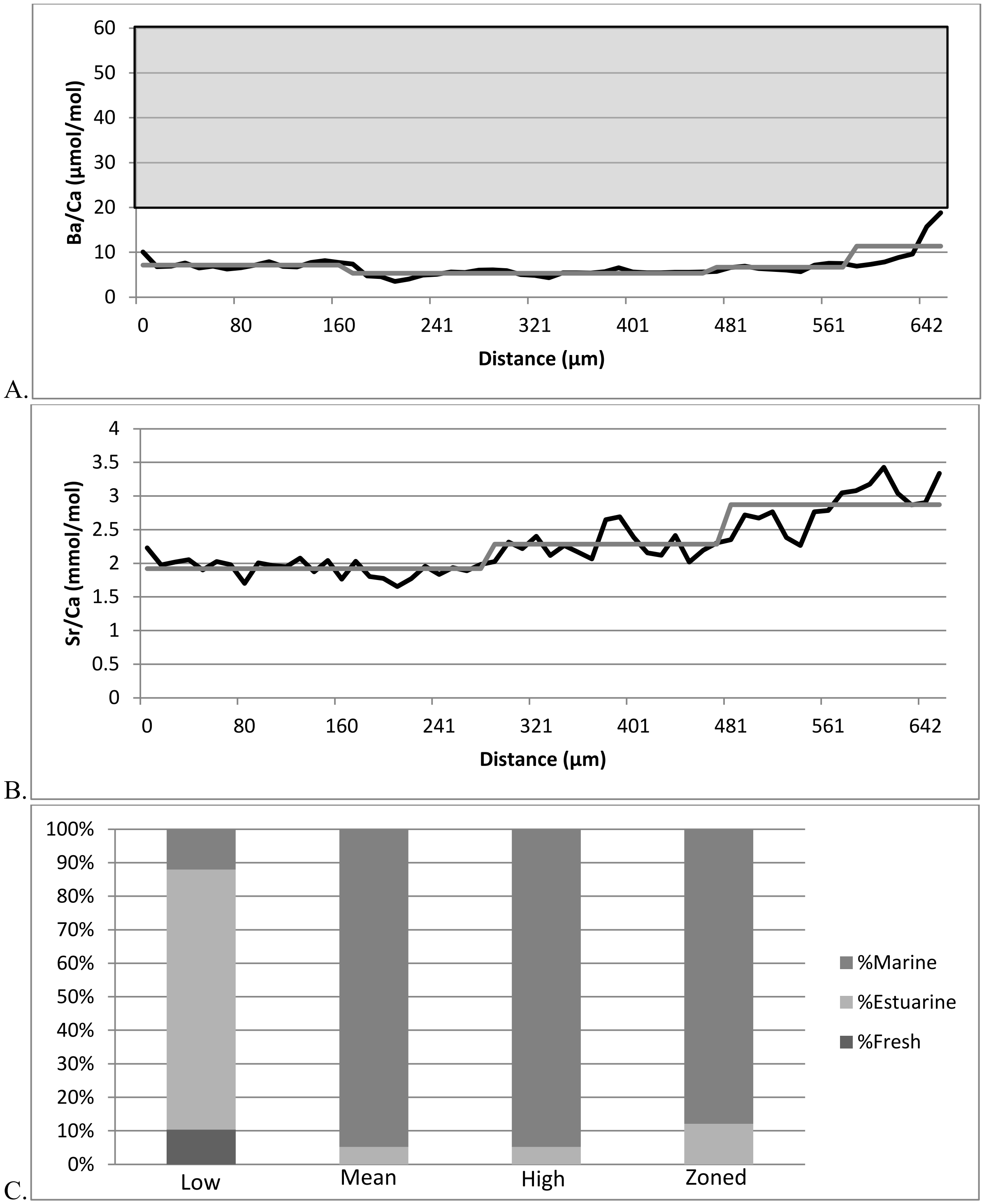


Figure AB.225. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 534. Figure 225.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

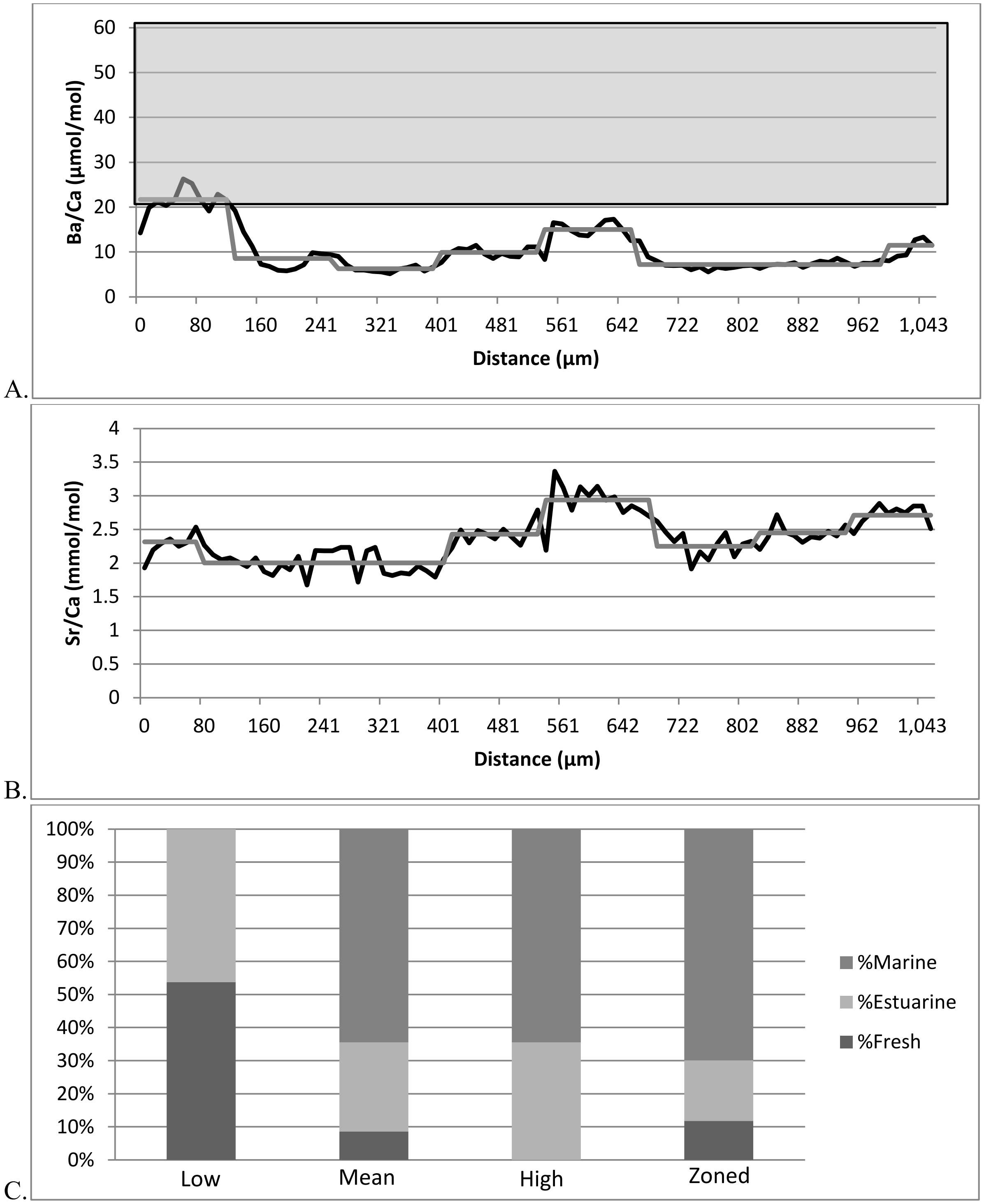


Figure AB.226. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 535. Figure 226.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

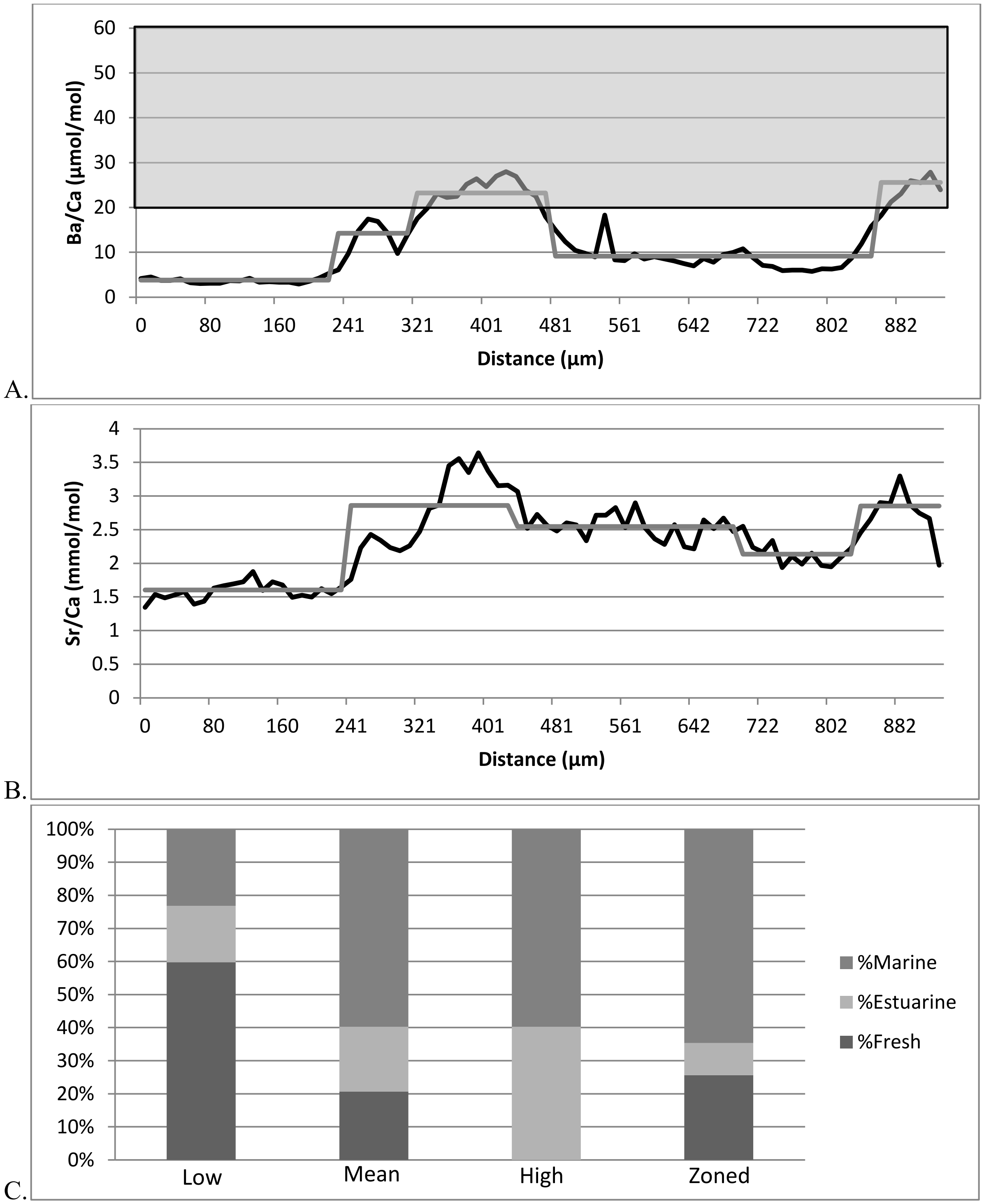


Figure AB.227. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 536. Figure 227.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

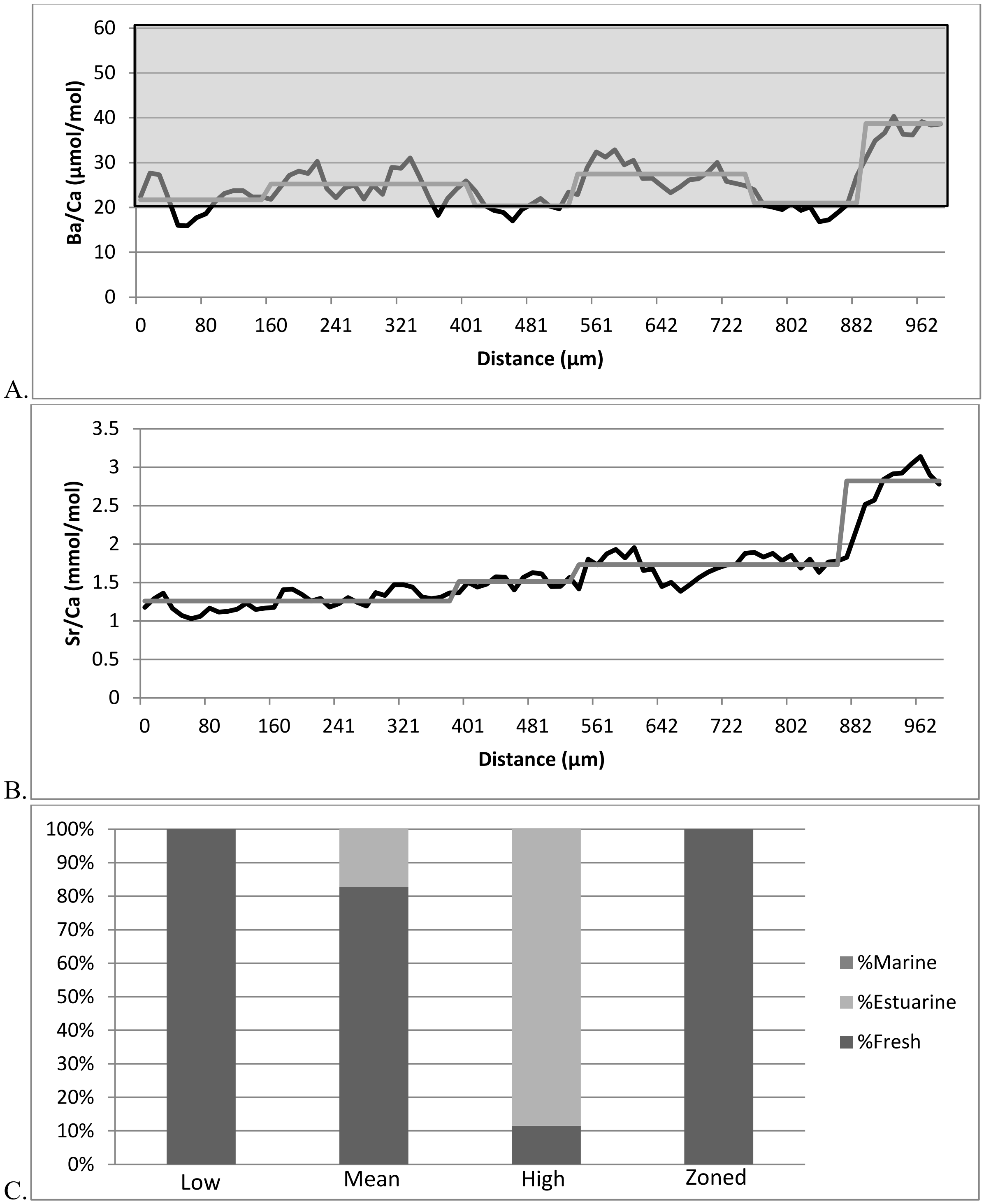
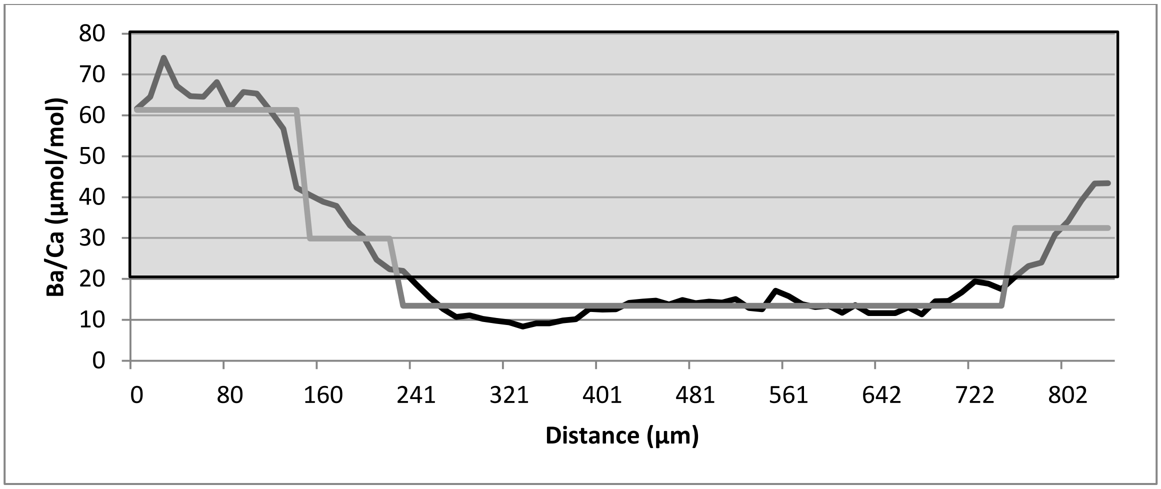
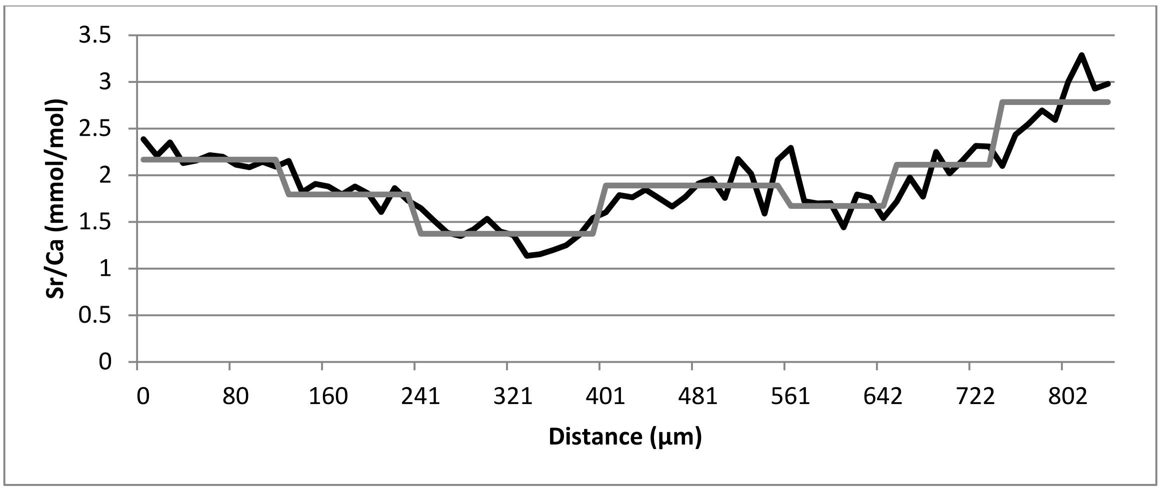


Figure AB.228. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 537. Figure 228.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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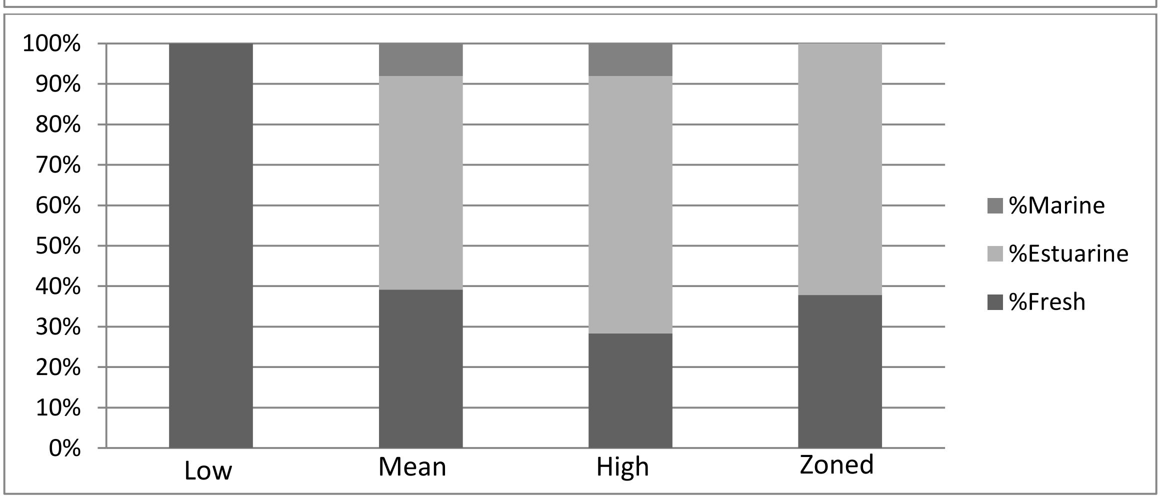
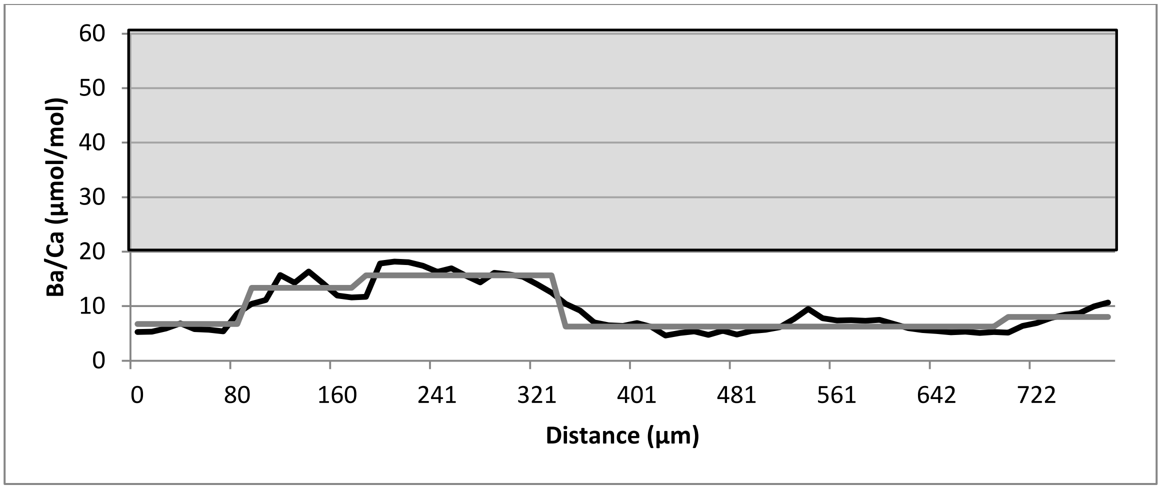
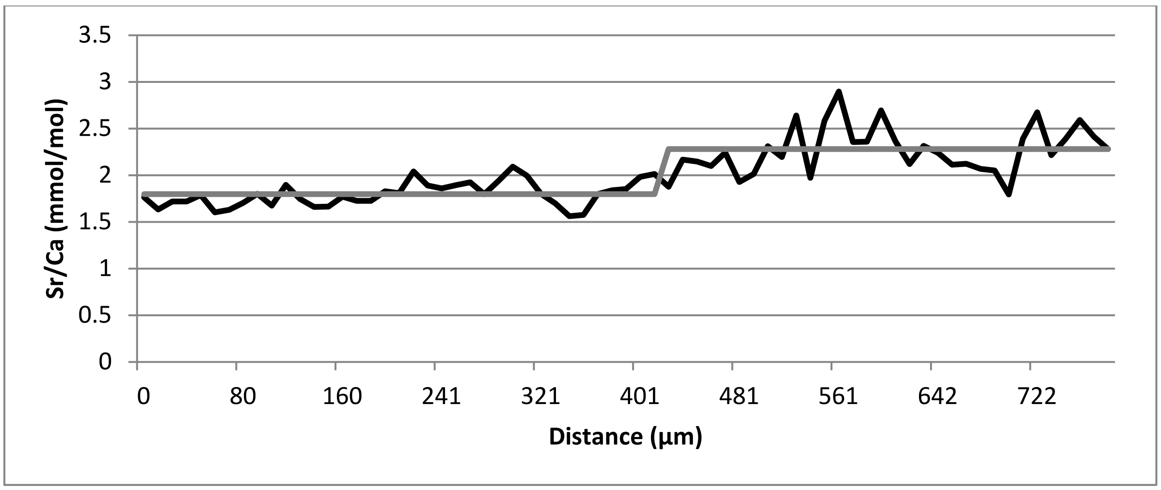


C.

Figure AB.229 The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 537. Figure 229.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.



A.



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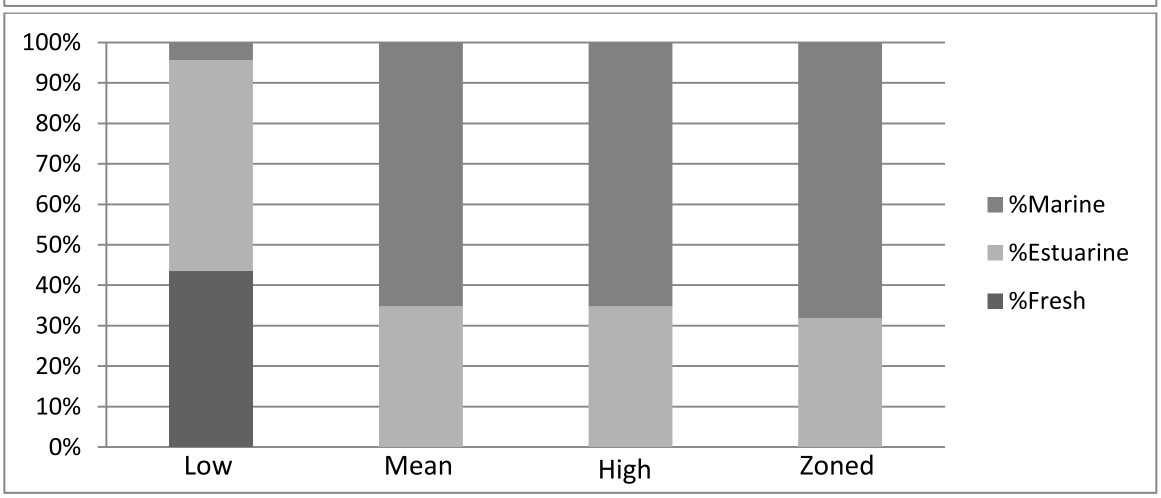

C.

Figure AB.230. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 540. Figure 230.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

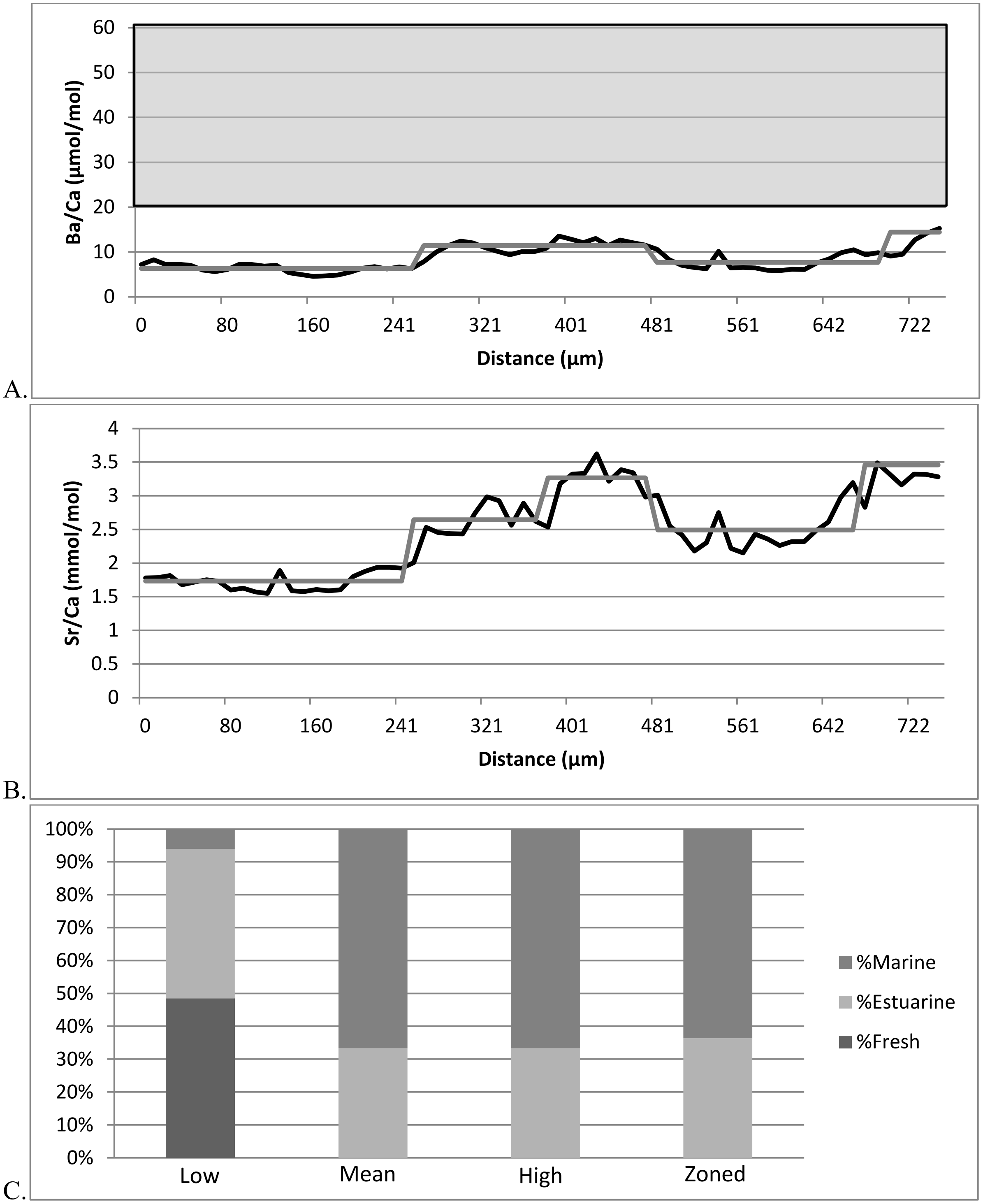

Figure AB.231. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 542. Figure 231.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

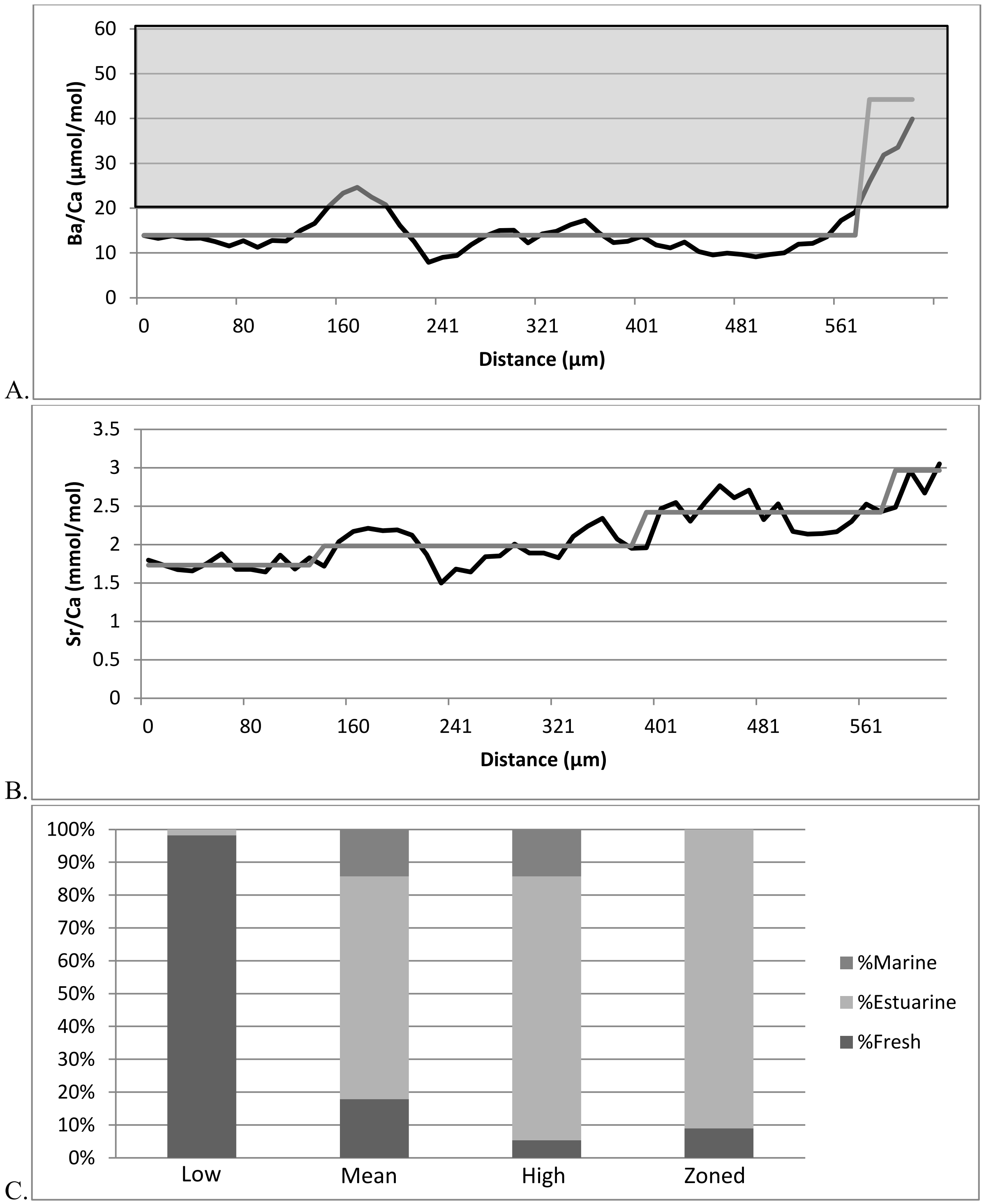

Figure AB.232. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 543. Figure 232.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

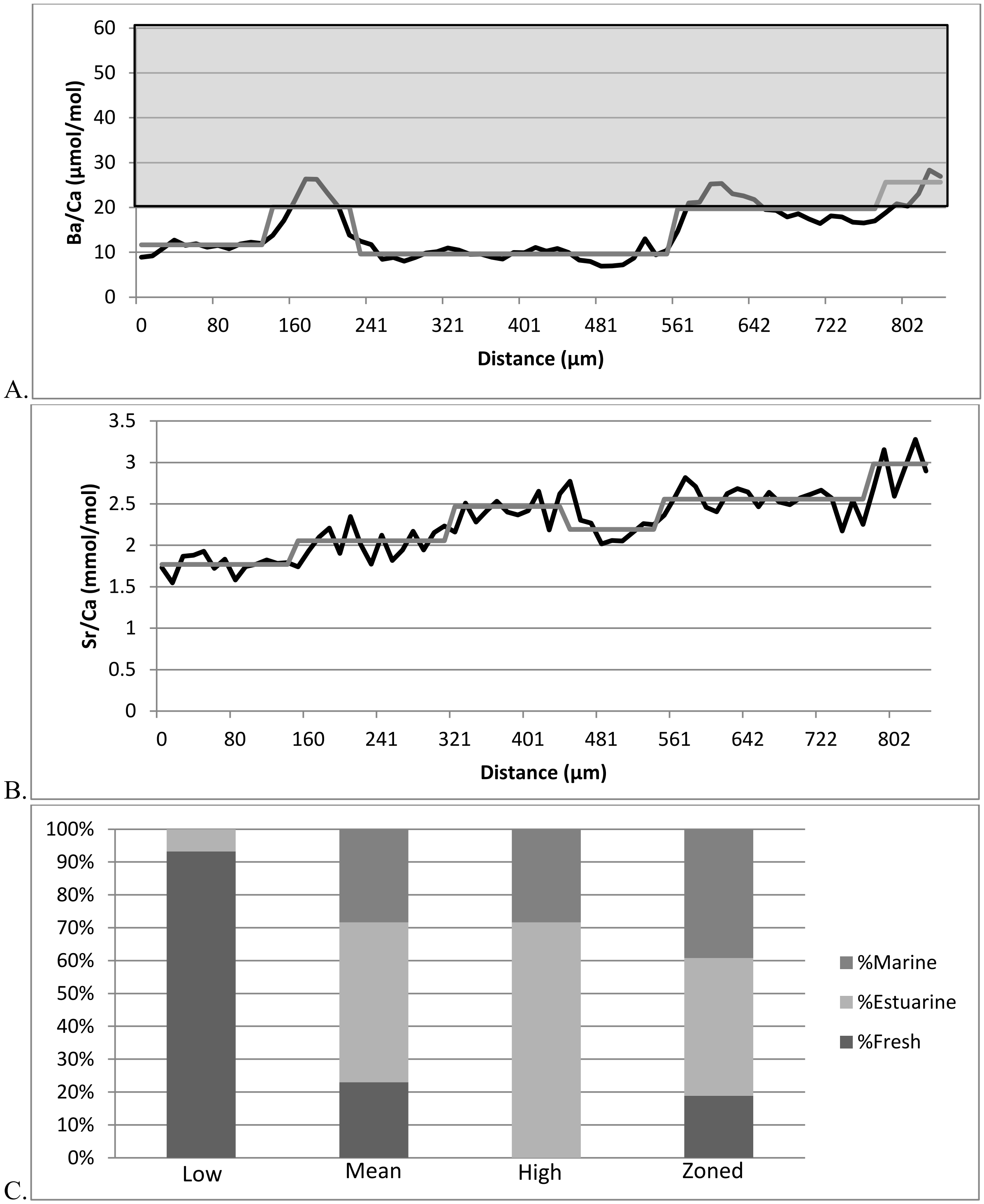

Figure AB.233. The Ba/Ca (A.) and Sr/Ca (B.) life history profile of individual 544. Figure 223.C. represents how the proportion of the life history transect that is classified as low salinity changes with the changing threshold values.

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Vita

Megan Katherine Nims was born in Walnut Creek, California, USA, the son of Douglas and Liz Nims. After graduating from Ottawa Hills High School in Toledo, Ohio in 2005, she entered the Ohio State University, Columbus, Ohio. Megan graduated from OSU in June of 2009 with a Bachelor of Arts in Evolution & Ecology and Spanish, with minors in Natural Resource Management and Security and Intelligence. In August of 2009, she entered the Master's degree program in the Department of Marine Science at the University of Texas at Austin.

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